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THE RÔLE OF NITROGEN IN THE PRODUCTION OF SPOTS IN WHEAT FIELDS[1]

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INTRODUCTION

During the past 20 years the Kansas Agricultural Experiment Station has devoted a great deal of attention to problems relating to soil nitrogen and has endeavored to follow any clue which it was felt might throw light on the relation of nitrogen to wheat production, particularly in regions of relatively little rainfall.

In the small-grain fields of Kansas there are frequently observed well-defined spots on which the grain is darker green and more vigorously growing than that on the surrounding areas. So abundant are these spots in the eastern half of Kansas that some fields present a polka-dot appearance, as shown in Figure 1. These spots are ordinarily 2 to 3 feet in diameter and circular or oblong in shape. In a typically spotted field when the grain is in the booting stage such spots are easily visible a half mile away. They are more frequently observed in fields that have been grazed, and are commonly attributed to deposits of feces or urine. They seem to occur upon heavy, potentially fertile soil as frequently as upon light, nonfertile soil, particularly if the season has been wet and cold. The general appearance of the spots, together with their supposed origin, indicated that they might in some way be connected with the nitrogen metabolism of the plant. The studies of Lipman,[2] who first called attention to these spots, confirmed this belief.

Some preliminary studies were conducted by the writers as early as 1916, and in the spring of 1929, 1930, and 1931 the studies were extended as far as it was felt the problem and facilities would justify. The object of these studies was to determine (1) the rôle, if any, that available nitrogen plays in the production of the spots; (2) in case available nitrogen were found to be a factor, to ascertain why more nitrogen became available in the soil of the spot than in the soil immediately adjacent to it; and (3) to measure the relative yield and quality of grain produced on the spot as compared with that on the field at large. A preliminary report of the 1929 investigations has already been presented.[3] The present paper gives in detail the results of the three seasons' studies.

[1] Received for publication, Dec. 28, 1931; issued August, 1932. Contribution No. 144 from the Department of Bacteriology and No. 211 from the Department of Agronomy, Kansas Agricultural Experiment Station.

[2] LIPMANN, C. B. THE NITRIFYING POWERS OF SOILS AS INDICES TO THEIR FERTILITY. Soc. Prom. Agr. Sci. Proc. 35 : 73-79. 1915.

[3] GAINEY, P. L., and SEWALL, M. C. INDICATIONS THAT AVAILABLE NITROGEN MAY BE A LIMITING FACTOR IN HARD WINTER WHEAT PRODUCTION. Jour. Amer. Soc. Agron. 22 : 639-641. 1930.

FIGURE 1.—Distant (A) and close (B) views of a typically spotted wheat field

METHODS

For these studies plant material and soil were collected from typical spots and from the area immediately adjacent to them (always within a radius of 10 feet). As Figure 2 indicates, the samples were taken from many fields over a wide territory. In all, 121 samples from 38 counties were collected and studied.

The samples taken from the spots are designated as G (good) and those taken from the field at large as P (poor). The exact technic followed during the three seasons varied somewhat; hence that employed in each year's work is described separately.

NITROGEN STUDIES

DATA FOR 1929

Most of the samples studied in 1929 were collected at some distance from Manhattan; hence laboratory studies could not be made for several days. This necessitated, in the case of soil to be analyzed for nitrate nitrogen, some treatment to prevent any change in the nitrate content during the interval between sampling and analysis. At the same time, it was desired to make certain bacteriological tests. These could not be conducted upon samples of soil to which a preservative had been added. To overcome these difficulties, two samples of soil were collected from a typical spot and two from the area immediately surrounding it. If the soil was dry enough to be handled with a soil tube, one was used, otherwise a garden trowel was employed. In either case the sample was a composite of a number of smaller samples, usually not less than six, taken to a depth of approximately 7 inches. The soil was placed in pasteboard cartons, and to one of the samples

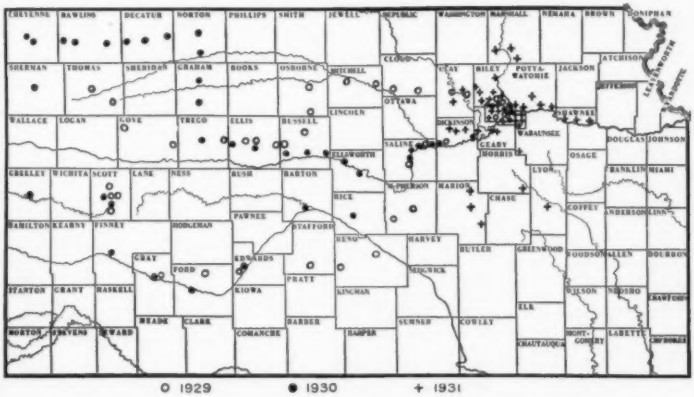

FIGURE 2.—Locations from which samples of plant material and soil were collected

a heavy application of toluol was added and mixed thoroughly with the soil. As soon as this sample reached the laboratory it was analyzed for nitrate nitrogen by the phenol-disulphonic acid method. The sample was then dried, ground, and analyzed for total nitrogen by the Kjeldahl method.

The sample that received no toluol was used for bacteriological studies. It was first passed through a coarse sieve, three meshes per centimeter, and the moisture content and water-holding capacity were then determined. Duplicate samples, the equivalent of 100 g of dry soil, were weighted into wide-mouth cotton-stoppered bottles and sufficient water added to bring the moisture content up to two-thirds saturation. These samples were held at room temperature for six weeks, the moisture lost through evaporation being replaced weekly. After six weeks' incubation the nitrate-nitrogen content was determined.

No special effort was made either in 1929 or the succeeding years to eliminate contamination completely. It was felt that, since there

was every opportunity for the transfer of soil from a spot to the surrounding area, or vice versa, under field conditions, the use of absolutely aseptic conditions would be a waste of effort. However, the instruments used in the field were freed of soil in passing from one sample to another, and in the laboratory the ordinary precautions were observed to prevent the gross transfer of material from one sample to another.

Soil samples were taken from 38 fields in 19 counties. From the same spot and surrounding area from which soil samples were taken, samples of the growing grain were also collected by pulling, thus including the crown and such roots as adhered. In 1929 the plants were not collected from a definite area as in 1930 and 1931. However, the number of plants was recorded, and comparisons are made on the basis of the individual plant. Such values give no indication as to the actual quantity of nitrogen removed from a unit area of soil, but since there was no marked difference in the stand of wheat on the spot and elsewhere, they do give relative values, or the comparison that is of special interest. After the soil had been washed from the roots and the number of plants recorded the entire batch of material was dried, ground, and analyzed for total nitrogen.[4]

The results of the 1929 studies are recorded in Table 1. The data here presented may be briefly summarized as follows:

TABLE 1.—*Data obtained from study of plants and soil taken from spots and from surrounding areas—1929*

Sample No.	Height of plants		Weight of plants		N in plants		N in soil		NO_3 in soil		NO_3 in incubated soil		NO_3 formed in incubated soil	
	P	G	P	G	P	G	P	G	P	G	P	G	P	G
	Ins.	*Ins.*	*Gms.*	*Gms.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*
1	6	28	1.48	3.76	1.21	2.38	.1350	.2020	9.7	17.5	88	266	78.3	248.5
2	9	28	2.01	5.38	1.00	1.85	.2160	.2180	9.2	8.5	175	313	165.8	304.5
3	5	21	1.68	3.05	.88	2.17	.0560	.0605	9.0	18.0	80	186	71.0	168.0
4	5	21	.56	2.67	1.06	1.74	.0760	.0810	8.7	8.5	63	105	54.3	96.5
5	6	15	.80	2.91	.98	2.31	.1025	.1035	7.5	9.0	82	110	74.5	101.0
6	10	27	1.08	5.67	1.73	2.24	.1030	.0990	20.2	26.7	90	104	69.8	77.3
7	5	18	.20	1.47	1.39	2.61	.1655	.1950	22.7	31.0	187	568	164.3	537.0
8	18	33	1.74	4.59	1.05	2.43	.1080	.1100	19.8	28.5	86	146	66.2	117.5
9	12	26	1.58	5.93	1.07	1.89	.1290	.1395	19.2	27.8	108	240	88.8	212.2
10	14	26	1.62	7.39	1.20	2.29	.1410	.1765	19.2	16.9	100	426	80.8	409.1
11	7	12	.27	.53	1.49	2.81	.1135	.1380	18.5	73.6	117	1,060	98.5	986.4
12	10	20	2.33	4.44	.82	2.15	.0330	.0360	17.0	120.7	61	114	44.0	−6.7
13	8	16	.25	.88	1.64	2.21	.1100	.1140	17.7	26.2	150	180	132.3	153.8
14	6	12	.60	.73	1.38	2.82	.1145	.1360	16.3	42.3	147	820	130.7	777.7
15	10	16	2.12	2.10	1.17	1.96	.1415	.1530	16.8	58.3	169	382	152.2	323.7
16	8	16	1.42	4.90	1.38	1.86	.1590	.1660	14.7	60.8	200	432	185.3	371.2
17	7	12	.44	.72	1.47	2.57	.1380	.1410	24.4	60.1	157	273	132.6	212.9
18	6	10	.33	.51	1.75	3.03	.1080	.1250	18.1	34.4	113	666	94.9	631.6
19	4	5	.28	.51	2.06	3.97	.1160	.1485	19.1	163.5	96	625	76.9	461.5
20	5	11	.09	.49	1.91	2.84	.1330	.1380	18.5	157.0	120	253	101.5	96.0
21	5	8	.31	.54	1.99	3.35	.1425	.1415	17.4	21.0	212	259	194.6	238.0
22	5	8	.32	.63	2.10	3.86	.1175	.1150	18.3	35.8	167	560	148.7	524.2
23	5	7	.22	.31	2.01	3.62	.1325	.1260	19.1	72.4	164	388	144.9	315.6
24	10	15	.72	1.18	1.43	2.87	.1730	.1865	18.0	58.4	165	840	147.0	781.6
25	11	17	1.92	5.58	1.65	2.50	.1405	.1480	18.3	73.2	156	152	137.7	78.8
26	10	16	1.52	2.53	1.30	2.69	.1405	.1580	15.9	23.2	114	802	98.1	778.8
27	10	14	.44	.49	1.53	2.65	.1685	.1885	16.2	67.9	286	498	269.8	430.1
28	8	16	.74	1.26	1.49	2.21	.1285	.1375	17.8	22.4	266	222	248.2	199.6
29	12	18	1.06	3.05	1.34	2.69	.1490	.1870	20.9	21.9	240	1,440	219.1	1,418.1
30	12	18	1.14	2.65	1.31	2.46	.1105	.1195	33.9	35.8	166	481	132.1	445.2
31	8	11	.40	.89	1.85	3.45	.1855	.2210	15.8	35.4	248	1,116	232.2	1,080.6
32	11	20	.59	1.36	1.26	2.94	.1605	.1780	118.5	146.5	260	1,346	141.5	1,199.5
33	9	20	.61	1.42	1.34	2.17	.1120	.1385	17.3	65.0	166	445	148.7	380.0
34	6	12	.24	.35	1.52	2.99	.1335	.1630	22.5	29.4	161	1,600	138.5	1,570.6
35	9	18	.28	1.04	1.33	3.01	.1235	.1510	20.6	73.7	172	1,200	151.4	1,126.3
36	8	14	.61	1.14	1.63	3.47	.1395	.1445	18.4	137.6	360	200	341.6	62.4
37	9	19	.47	2.57	1.45	2.53	.1455	.1565	16.0	31.7	136	400	120.0	368.3
38	10	24	.45	2.62	.99	2.40	.0960	.1075	16.1	49.1	158	453	141.9	403.9
Av.	8.4	17.1	.87	2.32	1.43	2.63	.1289	.1434	20.2	52.4	158	518	137.4	465.3

[4] The total nitrogen determinations on both soil and plant materials for 1929 and 1930 were made in the analytical laboratory of the Department of Chemistry under the supervision of Prof. W. L. Latshaw.

The average height of the G plants was 17.1 inches and of the P plants 8.4 inches, the former being taller than the latter in every comparison. The average weight of the G plants was 2.32 and of the P plants 0.87 g, there being only one instance in which the average weight of the P plant exceeded that of the G. In several instances, the exception just noted being one, there was an excessive number of plants within the spot, resulting in the absence of stooling and the development of slender, weak plants; otherwise the differences in weight between the P and G plants would have been even more marked.

The percentage of nitrogen in the P plants ranged from 0.82 to 2.10, with an average of 1.43, while the corresponding values for the G plants ranged from 1.74 to 3.97, with an average of 2.63. Without exception, the nitrogen content of the G sample exceeded that of the P sample. Since the average weight of the G plants was 2.67 times that of the P plants and the average nitrogen content of the former was 1.84 times that of the latter, the average quantity of nitrogen removed from the soil occupied by a G plant was 4.91 times that removed from the corresponding area occupied by a P plant.

Even though the G plants had removed 4.91 times as much nitrogen from the soil as had the P plants, the G soil when collected still contained, on an average, approximately 2.6 times as much nitrate nitrogen as did the P soil, the average NO_3 content being 52.4 and 20.2 p. p. m., respectively.

When the moisture content of the P and G soils was made up to optimum and the soils placed under identical incubation conditions for six weeks, the quantity of NO_3 present showed a marked increase in practically every sample and the quantity found in the G sample exceeded that in the P sample in 35 of the 38 comparisons, the average for P being 158 and for G, 518 p. p. m. If the NO_3 present originally be deducted from that present at the end of the six weeks' incubation, the quantity formed during the incubation period can be approximated. The average for the P samples is then found to be 137.4 and for the G samples 465.3 p. p. m. Obviously, the rate of accumulation of NO_3 in the G soil was relatively greater when the two were placed under identical conditions than it was in the field, for while the original NO_3 content of the G soil was 2.59 times that of the P soil, the accumulation of NO_3 in the G soil in the laboratory was 3.45 times that in the P soil. The difference in the accumulation under the two conditions can be partly explained by the more rapid absorption of NO_3 from the G soil under field conditions as a result of the heavier plant growth.

As would be expected in a group of soils collected from such widely scattered areas, there was a very marked variation in the nitrogen content of the different samples. A rather unexpected difference noted, however, was the uniformly higher nitrogen content of the G sample as compared with the P, there being only four exceptions to this rule. In many instances, the differences were not very great but the average was significant, being for the P and G soils 0.1289 and 0.1434, respectively, or 11 per cent higher for the G than for the P soils.

If the 38 comparisons are arranged in the order of the magnitude of the difference in nitrogen content between the P and G samples,

as has been done in Table 2, it is quite evident that, in spite of a number of marked exceptions, the excessive accumulation of NO_3 in G over P is definitely associated with the excess of nitrogen in G.

TABLE 2.—*Relationship between excess nitrogen in soil from spots and excess NO_3 accumulation (data from Tables 1 and 4), the comparisons being arranged in order of magnitude*

Sample No.	1929 data [a]		Sample No.	1931 data [b]	
	Excess N in G soil [c]	Excess NO_3 accumulation in G soil		Excess N in G soil [c]	Excess NO_3 accumulation in G soil
	Pounds per acre	*P. p. m.*		*Pounds per acre*	*P. p. m.*
1	1,340	170	20	654	926
29	760	1,199	32	608	524
10	710	328	5	516	664
31	710	848	37	486	611
19	650	385	31	474	540
7	590	373	18	420	261
34	590	1,432	39	382	325
35	550	975	16	376	537
33	530	231	19	370	180
11	490	888	2	368	78
14	430	647	22	368	224
27	400	160	7	348	328
26	350	681	33	292	421
32	350	1,058	8	284	17
18	340	537	10	284	371
24	270	635	25	264	157
15	230	172	27	264	100
38	230	262	9	224	183
37	220	248	13	216	191
9	210	123	4	206	140
28	180	−49	17	158	140
30	180	313	28	158	201
25	150	−59	29	146	64
16	140	186	11	142	−13
4	100	42	12	134	125
36	100	−279	34	128	95
20	100	−6	23	120	169
3	90	97	35	106	273
13	80	22	42	104	11
12	60	−51	21	98	6
17	60	80	14	82	30
2	40	139	40	82	114
8	40	51	6	60	63
5	20	27	26	60	−3
21	−20	43	43	50	35
22	−50	376	36	38	51
6	−80	8	3	38	63
23	−130	171	15	30	32
			24	22	57
			41	8	72
			1	−30	30
			30	−38	−8

[a] Correlation coefficient, 1929, 0.527 ± 0.079.
[b] Correlation coefficient, 1931, 0.843 ± 0.030.
[c] 2,000,000 pounds of soil.

The low correlation coefficient (0.527 ± 0.079), together with the fact that in all four instances in which the nitrogen content of P exceeded that of G the latter still exhibited a much higher NO_3 accumulation, indicates very strongly that some factor other than total nitrogen was influencing the rate of nitrate accumulation. However, in the 20 instances in which the G soil contained over 200 pounds per acre more nitrogen than did the P soil, the average excess accumulation of NO_3 was 568 p. p. m., ranging from +123 to +1,432 p. p. m.,

while the corresponding values for the 18 comparisons in which the difference in total nitrogen between P and G was less than 200 pounds per acre averaged 62 and ranged from −279 to +376 p. p. m. On the other hand, while the average total nitrogen was only 11 per cent more in the G than in the P soil, the average NO_3 accumulation during the six weeks' period was 239 per cent more in the G soil.

Flasks of Ashby's mannite solution were inoculated with both P and G soil, but the differences between the two soils in the type and quantity of growth that took place were so slight as to indicate that quantitative nitrogen determinations would yield no information of value; hence none were made. Where differences in growth between the P and G samples were evident they were, in most instances, in favor of the P samples.

DATA FOR 1930

During the spring of 1930 samples of soil and growing grain were collected from spots and from the immediately adjacent areas in 40 fields in 21 different counties. The methods used were similar to those employed in 1929, except that no sample of soil was taken for bacteriological studies and the growing grain was collected by cutting it just above the surface of the ground from a definite area, 1 or 2 square feet, depending upon the size and thickness of the plants.

The data for 1930 are presented in Table 3.

TABLE 3.—*Data obtained from study of plants and soil taken from spots and from surrounding areas in 1930*

Sample No.	Height of plants		Weight of plants		N in plants		N in soil		NO_3 in soil	
	P	G	P	G	P	G	P	G	P	G
	Inches	*Inches*	*Grams*	*Grams*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*P. p. m.*	*P. p. m.*
1	20	34	36.5	116.0	1.010	1.295	0.1120	0.1100	10.90	9.26
2	26	34	28.2	104.0	1.220	1.650	.1400	.1450	11.99	29.72
3	15	30	23.7	98.7	1.370	2.090	.2040	.2105	15.08	29.34
4	15	27	29.5	65.7	1.375	1.620	.1565	.1585	52.97	28.76
5	18	24	16.0		1.520		.1470	.1550	10.60	42.10
6	24	36	41.0	124.0	1.440	1.745	.1975	.2095	7.88	19.88
7	18	30	19.0	80.0	1.440	1.950	.1545	.1800	10.98	21.01
8	16	22	11.0	55.0	1.610	1.830	.1200	.1295	12.63	28.13
9	18	28	25.0	95.0	1.595	2.160	.1220	.1215	10.24	52.95
10	12	18	14.0	38.0	1.485	2.435	.1065	.1080	8.67	16.27
11	14	24	18.0	70.0	1.410	2.475	.0895	.1000	14.26	38.96
12	18	18	22.0	25.0	1.545	2.580	.0905	.1020	11.05	52.47
13	15	18	32.0	56.0	1.830	2.425	.1685	.1610	10.63	12.91
14	15	23	40.0	81.0	1.550	2.990	.1490	.2200	9.51	329.22
15	10	16	10.0	38.0	1.885	2.750	.1130	.1070	14.01	16.93
16	16	21	20.0	45.0	1.690	2.715	.1310	.1340	12.54	33.72
17	15	18	23.0	62.0	1.570	2.685	.1400	.1520	10.21	31.20
18	12	16	16.0	46.0	1.840	2.970	.2100	.2150	8.52	17.04
19	6	11	9.0	25.6	2.470	3.440	.0990	.1250	8.49	35.38
20	11	20	22.0	34.0	2.215	3.270	.1520	.1510	12.22	29.48
21	12	17	21.0	49.0	1.600	2.920	.1680	.1750	8.48	14.15
22	18	24	34.0	50.0	1.490	2.530	.1265	.1940	9.29	39.00
23	12	18	12.0	29.0	1.770	3.040	.1180	.1280	9.41	45.92
24	12	18	17.0	49.0	1.895	3.265	.1410	.1565	10.69	52.00
25	8	11	8.5	22.0	2.000	3.705	.1190	.1210	9.61	18.13
26	13	21	23.0	58.0	1.330	2.970	.1120	.1200	10.04	17.94
27	10	18	13.0	56.0	1.870	3.055	.1165	.1300	9.86	36.48
28	18	28	16.0	71.0	1.325	2.165	.1310	.1260	9.75	16.02
29	13	18	25.0	62.0	1.475	2.755	.1220	.1160	10.00	14.10
30	15	21	18.0	43.0	1.560	3.180	.1130	.1145	8.67	83.31
31	17	23	23.0	66.0	1.520	1.940	.1420	.1455	10.21	14.15
32	16	24	20.0	80.0	1.800	2.770	.1270	.1450	9.55	51.57
33	20	36	32.0	93.0	1.610	2.130	.1885	.1985	9.51	19.78

TABLE 3.—*Data obtained from study of plants and soil taken from spots and from surrounding areas in 1930*—Continued

Sample No.	Height of plants		Weight of plants		N in plants		N in soil		NO_3 in soil	
	P	G	P	G	P	G	P	G	P	G
	Inches	*Inches*	*Grams*	*Grams*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*P. p. m.*	*P. p. m.*
34	20	32	28.0	108.0	1.390	1.770	.1620	.1600	9 41	14 07
35	20	28	27 0	69.0	1.330	1.720	.1460	.1455	8.72	33.51
36	25	29	59.0	99.0	1.120	1.990	.1200	.1365	7.73	46.55
37	18	30	30.0	119.0	1.150	2.080	.1150	.1390	9.14	42.91
38	18	20	18.0	43.0	1.700	2.780	.1360	.1610	9.53	33.18
39	24	28	29.0	63.0	1.330	2.080	.1555	.1840	8.91	87.73
40	18	28	27.0	91.0	1.550	1.900	.1550	.1515	10.00	12.85
Average	16.02	23.5	23.41	66.30	1.572	2.457	.1379	.1486	11.297	39.20

In practically every instance the plants were taller and the dry weight greater from the spot than from the area immediately adjacent, the average values being as follows: For height, P 16 inches, G 23.5 inches; for dry weight, P 23.41 g and G 66.30 g. Furthermore, the percentage of nitrogen in the plants from the G soil was invariably greater than that in the plants from the P soil, the latter varying from 1.010 to 2.470 and averaging 1.572, while the former ranged from 1.295 to 3.705 and averaged 2.457. There were only three of the P samples in which the nitrogen content exceeded 1.9 per cent, whereas only seven of the G samples contained less than that amount. In 1929 there were five P samples with a nitrogen content in excess of 1.9 per cent and four G samples with a smaller percentage of nitrogen.

Since the average weight of dry matter per unit area from G was 2.83 times that from P and the nitrogen content of the plant material 1.56 times that of P, the G plants had absorbed 4.41 times as much nitrogen as the P plants growing upon equal areas. The corresponding value for 1929 was 4.91.

The average NO_3 content of the P soil was 11.3 and that of the G soil 39.2 p. p. m., the G exceeding the P in 38 of the 40 comparisons, the average NO_3 content of the G soil being 3.47 times that of the P soil. The corresponding figure for 1929 was 2.6.

The average nitrogen content of the 40 P soils was 0.1379 per cent and of the G soils 0.1485 per cent, as compared with 0.1289 and 0.1434 per cent, respectively, for 1929. No samples were incubated during the 1930 season.

DATA FOR 1931

In 1931, 43 samples were collected from 11 counties. All the samples were taken within a radius of 100 miles of Manhattan, which made it possible to initiate laboratory studies within a few hours after the samples were collected and to utilize the same sample for chemical and bacteriological studies. Nitrate and total nitrogen analyses of the soil were run as in previous years. After moisture content and water-holding capacity were determined, duplicate 100 g (dry basis) samples were weighed into wide-mouth cotton-stoppered bottles, and the moisture was made up to two-thirds saturation and held at room temperature for four weeks, the moisture lost being replaced weekly. Two additional samples were treated in the same way, except that 1 g of cottonseed meal containing 7 per cent nitrogen was thoroughly

mixed into the soil. All four samples were analyzed for nitrate nitrogen after four weeks' incubation.

The nitrogen-fixing ability of the various soils was determined by inoculating (in quadruplicate) poured plates containing 20 c c of carefully washed mannite agar with 1 g of soil, immediately analyzing two for total nitrogen and incubating the remaining two at 28° C. two weeks, after which the nitrogen content was determined. During incubation the plates were examined at frequent intervals and the relative growth of Azotobacter recorded.

The growing grain was cut from an area of 2 square feet, dried, weighed, ground, and analyzed for total nitrogen.

The 1931 data are presented in Table 4. In every one of the 36 comparisons there shown the height and dry weight of plants from the spot exceeded that from the surrounding area, the average height being P 11.9 and G 20.7 inches, and average weight P 39.5 and G 114.9 g. The nitrogen content of the P plants ranged from 0.80 to 2.83 per cent and averaged 1.55 per cent, while that of the G material varied from 1.22 to 4.10 per cent and averaged 2.56 per cent. There were only three P samples in which the nitrogen content was higher than 1.9 per cent and only four G samples in which the nitrogen content was lower than that amount.

TABLE 4.—*Data obtained from study of plants and soil taken from spots and from surrounding areas—1931*

Sample No.	Height of plants		Weight of plants		N in plants		N in soil		NO_3 in soil		NO_3 in incubated soil		NO_3 formed in incubated soil		NO_3 in incubated soil containing [a]		NO_3 formed from [a]		Nitrogen fixed per plate	
	P	G	P	G	P	G	P	G	P	G	P	G	P	G	P	G	P	G	P	G
	Inches	*Inches*	*Gms.*	*Grams*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*Milligrams*	*Milligrams*
1							0.1200	0.1185	10.0	10.0	51.0	81.4	41.0	71.4	1,014.0	952.0	963.0	870.6	3.37	3.86
2							.1631	.1815	10.3	10.7	88.3	166.6	78.0	155.9	1,144.0	1,153.0	1,055.7	986.4	−.04	.23
3							.1571	.1590	3.2	3.1	50.4	113.7	47.2	110.6	1,156.0	1,210.0	1,105.6	1,096.3	.15	.27
4							.1433	.1536	4.5	3.2	56.8	195.9	52.3	192.7	1,015.0	1,864.0	958.2	1,668.1	1.05	5.13
5	9	20	29.5	90.5	1.94	3.34	.1883	.2141	9.7	50.7	70.0	775.0	60.3	724.3	1,085.0	1,226.0	1,015.0	451.0	3.87	3.56
6	8	13	17.6	51.2	1.56	2.68	.1324	.1354	42.1	15.9	58.8	95.7	16.7	79.8	1,056.0	1,212.0	997.2	1,116.3	−.37	−.11
7	8	11	35.4	62.3	1.68	2.99	.1270	.1444	7.7	99.3	52.8	472.0	45.1	372.7	776.0	1,036.0	723.2	564.0	3.80	3.44
8							.1358	.1500	7.8	342.7	46.5	398.0	38.7	55.3	1,036.0	1,224.0	989.5	826.0	3.83	3.61
9							.1268	.1380	6.1	27.5	67.1	271.0	61.0	243.5	982.0	1,486.0	914.9	1,215.0	.82	.11
10	6	15	34.1	82.1	1.75	2.96	.1669	.1811	6.7	86.6	49.5	500.0	42.8	413.4	1,349.0	1,945.0	1,299.5	1,445.0	4.13	3.71
11	11	18	44.1	119.8	2.83	2.65	.1838	.1909	16.7	21.3	127.5	119.0	110.8	97.7	732.0	1,196.0	604.5	1,077.0	.16	.03
12	8	14	25.0	68.0	2.24	4.10	.1234	.1301	9.3	158.2	72.7	346.3	63.4	188.1	1,303.0	1,748.0	1,230.3	1,401.7	2.88	3.29
13							.1508	.1616	9.6	140.2	68.8	390.6	59.2	250.4	1,328.0	1,690.0	1,259.2	1,299.4	3.86	4.12
14	8	16	37.0	118.5	1.62	2.62	.1699	.1740	4.5	10.5	63.9	99.5	59.4	89.0	1,265.0	1,371.0	1,201.1	1,271.5	4.35	3.64
15	8	10	19.5	45.1	1.72	2.95	.1665	.1680	5.7	12.2	98.0	136.8	92.3	124.6	942.0	1,169.6	844.0	1,032.8	.21	−.04
16	7	11	21.7	74.2	1.51	3.01	.1695	.1883	6.2	64.8	80.7	676.0	74.5	611.2	1,182.0	936.8	1,101.3	260.8	2.78	3.90
17	13	26	43.4	179.5	1.55	2.30	.1796	.1875	16.5	22.3	99.4	245.4	82.9	223.1	1,224.0	1,666.0	1,124.6	1,420.6	4.28	3.90
18	12	22	35.5	122.0	1.65	2.75	.1451	.1661	1.7	16.9	81.0	357.3	79.3	340.4	1,156.0	1,038.6	1,075.0	681.3	.22	1.31
19	10	16	41.4	82.0	1.85	3.18	.1585	.1770	3.4	147.0	117.8	441.8	114.4	294.8	1,480.0	1,494.0	1,362.2	1,052.2	1.57	.69
20	10	18	20.5	73.4	1.79	2.71	.1376	.1703	5.3	156.3	106.7	1,184.0	101.4	1,027.7	1,065.5	1,962.0	958.8	778.0	4.75	3.94
21	10	17	32.4	90.7	1.50	2.32	.1564	.1613	4.2	4.4	99.7	105.6	95.5	101.2	1,028.8	1,153.0	929.1	1,047.4	.41	.45
22	15	22	52.2	105.2	1.54	2.38	.1125	.1309	3.9	27.6	47.4	294.9	43.5	267.3	819.0	1,030.0	771.6	735.1	.98	.30
23	14	24	75.2	195.7	1.35	2.28	.1808	.1868	5.6	96.0	91.0	350.1	85.4	254.1	1,046.0	968.0	955.0	617.9	1.13	.77
24	12	18	27.7	72.7	1.48	2.62	.1568	.1579	6.7	15.4	61.8	127.0	55.1	111.6	980.0	894.0	918.2	767.0	1.29	1.42
25	13	20	46.0	107.1	1.44	2.71	.1436	.1568	4.8	25.1	48.5	226.2	43.7	201.1	786.2	1,179.0	737.7	952.8	4.38	3.97
26	8	12	14.6	34.1	1.84	3.76	.1215	.1245	4.0	31.4	71.1	95.7	67.1	64.3	1,125.0	1,407.3	1,053.9	1,311.6	.56	.41
27	11	16	32.1	80.1	1.75	2.89	.1496	.1628	3.8	32.7	74.4	203.5	70.6	170.8	784.0	1,376.0	709.6	1,172.5	5.09	2.41
28	12	18	42.4	90.4	1.63	2.91	.1459	.1538	4.8	47.1	77.3	320.9	72.5	273.8	1,323.0	1,657.0	1,245.7	1,336.1	3.89	3.90
29	10	24	36.0	193.0	1.43	2.26	.0955	.1028	2.5	6.4	54.5	122.8	52.0	116.4	1,547.0	1,444.0	1,492.5	1,321.2	4.55	5.17
30	12	26	67.0	214.1	1.34	1.54	.1294	.1275	3.2	3.1	63.1	55.1	59.9	52.0	1,075.8	1,317.0	1,012.7	1,261.9	2.39	.75
31	15	30	89.9	170.7	1.33	2.71	.1376	.1613	4.7	109.0	61.8	705.9	57.1	596.9	1,337.0	1,140.6	1,275.2	434.7	5.36	4.76
32	9	26	28.7	212.2	1.54	2.44	.0611	.0915	3.1	24.4	38.7	583.8	35.6	559.4	1,462.6	1,524.0	1,423.9	940.2	4.64	4.88
33	17	31	31.7	219.7	1.25	2.12	.1305	.1451	3.9	21.8	102.9	541.8	99.0	520.0	1,075.2	1,720.0	972.3	1,178.2	3.79	3.90
34	15	34	24.2	139.5	.95	1.75	.0379	.0443	1.4	5.8	34.1	133.7	32.7	127.9	523.0	336.0	488.9	202.3	3.56	3.66
35	14	29	24.5	129.7	1.72	2.07	.1245	.1298	3.4	43.4	77.9	391.2	74.5	347.8	1,306.0	1,280.0	1,228.1	888.8	1.05	−.22
36	20	30	60.4	188.0	1.35	1.55	.1534	.1553	3.2	4.0	113.6	165.4	110.4	161.4	1,600.0	1,526.0	1,486.4	1,360.6	4.04	4.27
37	18	22	83.2	103.9	1.29	2.23	.2063	.2306	5.2	48.0	163.5	816.8	158.3	768.8	1,139.0	2,025.0	975.5	1,208.2	−.30	−.08
38	11	25	27.9	143.8	1.28	1.94	.1875	.1946	4.0	9.2		555.2		546.0	962.0	1,396.0		840.8	.27	.00
39	11	24	34.2	82.7	1.72	2.42	.1365	.1556	4.4	26.5	92.8	440.0	88.4	413.5	892.0	1,381.0	799.2	941.0	2.00	.18

40	9	18	15.6	88.0	1.61	2.88	.1508	.1549	3.0	5.9	77.9	195.2	74.9	189.3	1,536.0	1,402.0	1,458.1	1,206.8	3.89	5.24
41	16	20	30.2	79.7	.80	2.51	.1473	.1477	6.5	53.4	78.2	196.8	71.7	143.4	1,145.0	1,069.0	1,066.8	872.2	04	−.39
42	24	30	97.8	150.5	.92	1.22	.1324	.1376	3.2	3.7	61.8	72.8	58.6	69.1	958.1	1,168.0	896.3	1,095.2	−.13	−.30
43	15	20	43.7	76.6	1.17	2.36	.1819	.1844	4.1	4.0	104.8	139.6	100.7	135.6	1,145.0	1,129.0	1,040.2	989.4	−.38	−.38
Average	11.9	20.7	39.5	114.9	1.55	2.56	.1447	.1555	6.5	47.6	76.3	323.4	69.7	275.8	1,113.6	1,327.9	1,040.9	1,004.5	2.28	2.18

[a] Cottonseed meal 1 g per 100 g of soil.

Since the average weight of dry plant material from equal areas was 2.91 times as much from the G as from the P soil and the nitrogen content 1.65 times as great in the G soil, there had actually been removed, per unit area, 4.80 times as much nitrogen from the G soil as from the P. The corresponding values for 1929 and 1930 were 4.91 and 4.41, respectively. In spite of the fact that 4.80 times as much nitrogen had been removed from the G as from the P soil, the soil from the spots still contained an average of 47.6 p. p. m. NO_3 as compared with an average of 6.5 p. p. m. in the surrounding soil. There were only 5 of the 43 comparisons in which the NO_3 content of the P soil exceeded that of the G. In one case they were the same.

When the moisture content of the soils was brought to optimum and the two held under identical conditions of temperature, aeration, etc., for four weeks, a very rapid accumulation of NO_3 took place in practically all samples, the accumulation being approximately four times as rapid in the G soil as in the P. The average quantities of NO_3 found after incubation were 76.3 p. p. m. for the P samples and 323.4 for the G samples, the quantities formed during the 4-week period being on an average 69.7 p. p. m. in the the P soil and 275.8 in the G soil. These comparative values, together with the almost identical results obtained in 1929, lead inevitably to the conclusion that there must have been present in the G soil either a much more active nitrifying flora or a quantity of nitrogen capable of being much more easily transformed into the nitrate condition. If the differences in favor of the G soil were due to a more active microflora, then when an abundance of easily transformed nitrogen was added to the two soils in equal quantities the microflora of G should be capable of transforming the added nitrogen into NO_3 at a much more rapid rate, whereas, if no difference existed in the two microfloras, the rate at which the added nitrogen would be transformed into nitrate nitrogen should be approximately the same.

A comparison of the nitrate content of the P and G soils to which cottonseed meal was added shows the NO_3 content of the latter to be appreciably higher than that of the former, the average values being 1,113.6 and 1,327.9 p. p. m., respectively, for P and G. However, if the NO_3 present in the incubated soil not receiving cottonseed meal be subtracted from that present in samples receiving the added nitrogen, then an approximation of the nitrate nitrogen actually formed from the added nitrogen is reached. When this is done it is found that the values are on an average practically identical, being for the P soil 1,040.9 and for the G soil 1,004.5 p. p. m. Furthermore, in 22 of the 42 comparisons the P soil exceeded the G, while in the other 20 the reverse was true. This is almost conclusive evidence that the difference in the accumulation of NO_3 is not connected with a more efficient microflora in the soil of the spots, but is in some way associated with differences in the quantity or quality of the nitrogen content.

Again, as in the two preceding seasons, the average nitrogen content of the G samples was found to be slightly higher than that of the P samples, the actual values being 0.1447 and 0.1555 per cent. In only two comparisons did the nitrogen content of the P sample exceed that of the G.

If the soils are arranged in the order of the magnitude of the difference in nitrogen between the P and G samples, as was done for the

1929 data, and these differences compared with those in nitrate accumulation during the 4-week incubation period, it will be noted that the two are associated. (Table 2.) The coefficient of correlation is 0.843 ± 0.030, as compared with 0.527 ± 0.079 for 1929, a significant correlation. The association is more strikingly shown if the average NO_3 accumulation of those soils in which the difference in nitrogen content exceeded 200 pounds is compared with that of the soils in which the difference was less marked. In the former group the average excess NO_3 accumulation in G over P is 339 p. p. m., and in the latter only 74 p. p. m., as compared with 568 and 62 for the 1929 data.

In spite of definite indications that the higher nitrate content of the G soil is associated with the higher total nitrogen content, the large number of instances, both in the 1929 and 1931 data, in which P samples with a relatively high total nitrogen content showed relatively low NO_3 accumulation, and G samples with relatively low total nitrogen content showed high NO_3 accumulation, indicate that the total nitrogen alone can not account for the difference in the rate of nitrate accumulation. There are comparatively few instances in which the maximum accumulation of NO_3 in the P soil, regardless of the total nitrogen content, has equaled the minimum accumulation in the G samples. A concrete illustration will suffice to emphasize this point. Samples 34 and 32 contained the lowest percentage of nitrogen of any of the G soils in the 1931 series, both being lower than any P sample except their corresponding numbers, yet the accumulation of NO_3 in the 34 G sample was exceeded by that in only 1 P sample, and the accumulation of NO_3 in the 32 G sample was 3.5 times the highest accumulation in any P sample.

That there must be a difference in the quality as well as the quantity of the nitrogen present in the G soil as compared with that in the P soil is further substantiated by the following comparison: On an average, the G soil contained only 7.5 per cent more nitrogen than the P soil, yet during the 4-week incubation period the G soil formed 296 per cent more NO_3. The corresponding values for the 1929 data were 11 and 239 per cent.

A second attempt was made in 1931 to measure the relative nitrogen-fixing ability of the soil from spots and from the soil immediately surrounding them. Poured plates of carefully washed mannite agar were inoculated with a definite quantity of soil, incubated for two weeks, and the quantity of nitrogen determined. Colony counts were also made at frequent intervals. On the whole, no significant difference was found in the quantity of nitrogen fixed, the average for the 43 comparisons being 2.28 and 2.18 mg per plate for the P and G samples, respectively. In a few instances significant differences were observed, the difference being sometimes in favor of one sample and sometimes in favor of the other. There was, however, a rather striking difference between the two samples in the early development of Azotobacter colonies. Approximately one-half the soils failed to show appreciable development of the bacteria, Azotobacter being either completely absent or confined to a few isolated colonies, possibly arising from contaminations. In a few instances the development of colonies was quite similar in both samples, but in most cases there was a striking difference in favor of the P soil. After Azotobacter had grown for two weeks, the total mass was approximately

equal in the two samples; hence no marked difference in nitrogen fixation was evident. The reason for the presence of larger numbers of Azotobacter in the P soil is not clear. It is possible that the growth of Azotobacter in the presence of an abundance of available nitrogen, such as occurred in the soil of the G spots, resulted in a temporary lowering or loss of nitrogen-fixing ability and that when the bacteria were transferred to a pabulum practically devoid of combined nitrogen many were unable to survive. However, those that did survive soon regained their nitrogen-fixing ability and thrived.

YIELD STUDIES

DATA FOR 1929

Because of the wide distribution of fields from which soil samples were studied and the very short time in which samples of mature grain could be collected, it was impossible to obtain yield data from the same fields from which the soil samples were taken. Comparisons of yield have been confined to four counties, Riley, Clay, Dickinson, and Geary. For the season of 1929 samples of ripe grain were harvested from spots and the area immediately adjacent in 20 wheat and 2 oat fields. The same four drill rows were cut for a distance of 2 feet in the spot and immediately adjacent to the spot. In some instances the cutting was actually continuous, and in no instance was it separated by more than 2 feet. The harvested area approximated 4 square feet, or 0.0001 acre. The 1929 data are recorded in Table 5.

TABLE 5.—*Yield of wheat or oat grain from equal areas of 4 square feet, P and G soil, in 1929*

Sample No.	Yield (grams) of grain on—		Sample No.	Yield (grams) of grain on—		Sample No.	Yield (grams) of grain on—	
	P area	G area		P area	G area		P area	G area
1	48.45	97.47	9	51.36	115.46	17	50.13	108.44
2	35.56	117.91	10	36.65	144.69	18	50.80	143.65
3	42.38	108.59	11	55.71	110.14	19	51.12	108.10
4	55.59	138.38	12	45.94	123.65	20	66.85	167.54
5	28.14	165.49	13	44.07	96.38	21	50.28	110.52
6	30.82	146.52	14	17.51	77.62	22 [a]	52.06	222.44
7	50.25	138.88	15 [a]	16.56	146.17			
8	43.35	96.10	16	47.23	121.79	Average	[b]44.13	[c]127.54

[a] Oats.
[b] 10.89 per cent protein in composite sample, oat samples not included.
[c] 12.37 per cent protein in composite sample.

The average yield of wheat and oats from the P areas was 44.13 g and from the G areas 127.54 g, the latter being 2.89 times the former. A composite of the wheat samples was analyzed for total nitrogen and found to contain 1.91 and 2.17 per cent, respectively, for the P and G soils, or 10.89 and 12.37 per cent protein.

DATA FOR 1931

In 1931 samples of mature grain were harvested, in the same manner as in 1929, from P and G areas in 27 fields. The weight of grain, percentage of nitrogen in each sample, and percentage of protein calculated from the nitrogen are presented in Table 6. The essential points to be noted from the data in this table are that the yield of

grain from the G areas average 2.12 times that from equal P area, and contained an average of 29.19 per cent increase in protein content. The corresponding values for 1929 were 2.89 and 13.59, respectively. In only 2 of the 27 comparisons did the grain from the P area contain as much nitrogen as that from the G area, while the maximum excess nitrogen in grain from a G area over that in grain from a P area was 96 per cent.

TABLE 6.—*Yield and protein content of wheat grain from equal areas, 2 square feet, of P and G soil in 1931*

Sample No.	Weight of grain		Nitrogen in grain		Protein content of grain		Increase or decrease in protein in grain from G soil over that in grain from P soil [a]
	P	G	P	G	P	G	
	Grams	*Grams*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
1	33.0	122.5					
2	108.0	228.0	1.85	2.01	10.55	11.46	8.63
3	66.5	212.0	1.67	2.19	9.52	12.48	31.09
4	93.0	143.0	1.85	2.19	10.55	12.48	18.29
5	53.0	150.5	1.63	2.22	9.29	12.65	36.16
6	43.0	102.0	1.86	2.42	10.60	13.79	30.09
7	25.0	84.0	2.12	2.36	12.08	13.45	11.34
8	57.0	127.0	1.73	2.12	9.86	12.08	22.52
9	89.0	169.0	1.78	2.39	10.15	13.62	34.19
10	96.0	147.5	1.76	2.53	10.03	14.42	43.77
11	61.0	182.0	1.73	2.28	9.86	13.00	31.85
12	40.5	132.0	1.30	2.30	7.40	13.11	77.16
13	82.0	117.0	1.68	2.25	9.58	12.83	33.92
14	87.0	119.0	1.43	1.72	8.15	9.80	20.25
15	64.0	112.0	1.25	2.45	7.13	13.96	95.79
16	49.5	114.0	1.87	2.01	10.66	11.46	7.50
17	26.0	63.0	1.87	2.50	10.66	14.25	33.68
18	65.5	119.5	1.70	2.46	9.69	14.02	44.68
19	81.5	166.0	1.60	2.64	9.12	15.05	65.02
20	80.0	161.5	1.85	1.79	10.54	10.20	−3.23
21	43.0	113.0	1.90	2.30	10.83	13.11	21.05
22	53.0	132.0	1.92	2.00	10.94	11.40	+4.20
23	59.0	101.0	1.76	2.46	10.03	14.02	39.78
24	52.0	110.0	1.94	2.37	11.06	13.51	22.15
25	65.0	95.5	2.24	3.68	12.77	20.98	64.29
26	52.0	104.0	2.12	2.55	12.08	14.54	20.36
27	47.0	109.0	2.62	2.59	14.93	14.76	−1.14
Average	61.9	131.0	1.81	2.34	10.31	13.32	29.19

[a] Percentage increase or decrease in percentage of protein.

DISCUSSION

From a determination of the quantity of growth on equal areas within and without typical spots and an analysis of the plant material for total nitrogen, information was obtained relative to the actual quantities of nitrogen utilized under the two conditions. Since the quantity of a particular plant-food element available in the soil may influence the percentage of that element in the plant, as well as the total quantity absorbed, the quantitative analysis of the plant material was doubly important. As a result of these determinations it was found that, with one possible exception, the dry weight of plant material produced per unit area in the 113 comparisons was greater in the spot than in the area immediately adjacent (Table 7), the average values of G for the three years being, respectively, 2.67, 2.83, and 2.91 times those of P.

TABLE 7.—*Summary of data obtained in 1929, 1930, and 1931*

Item	Average height of plant		Average weight of plant material		Average nitrogen in plants		Average nitrogen in soil		Average NO_3 in soil	
	P	G	P	G	P	G	P	G	P	G
	In.	*In.*	*Grams*	*Grams*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*P. p. m.*	*P. p. m.*
1929 [a]	8.4	17.1	0.87	2.32	1.43	2.63	0.1289	0.1434	20.2	52.4
1930 [b]	16.0	23.5	23.41	66.30	1.57	2.46	.1379	.1485	11.3	39.2
1931 [c]	11.9	20.7	39.50	114.90	1.55	2.56	.1447	.1555	6.5	47.6
Total comparisons, number	114		113		113		121		121	
Pairs in which G exceeded P, number	113		112		112		104		111	
Comparisons in which G exceeded P, per cent	99		99		99		86		92	

Item	Average NO_3 in incubated soil		Average NO_3 formed during incubation		Average NO_3 in incubated soil containing cottonseed meal		Average NO_3 formed from cottonseed meal during incubation		Average nitrogen fixed, per plate	
	P	G	P	G	P	G	P	G	P	G
	P.p.m.	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*Milligrams*	*Milligrams*
1929 [a]	158.0	518.0	137.4	465.3						
1930 [b]										
1931 [c]	76.3	323.4	69.7	275.8	1,113.6	1,327.9	1,040.9	1,004.5	2.28	2.18
Total comparisons, number	80		80		43		42		43	
Pairs in which G exceeded P, number	75		72		30		20		20	
Comparisons in which G exceeded P, per cent	94		90		70		+48		47	

[a] Weight of plant material based upon individual plant.
[b] Weight of plant material from 1 square foot.
[c] Weight of plant material from 2 square feet.

In the 113 comparisons the percentage of nitrogen found in the G material was greater than that in the P material in all instances except one, the actual average percentage of nitrogen being 1.84, 1.57, and 1.65 times as great in G as in P. The calculated quantity of nitrogen absorbed from the soil per unit area was, at the time the plant material was collected, 4.91, 4.41, and 4.80 times as great for G as for P for the three years under study. These same differences were carried through the grain to maturity, for the total quantity of nitrogen found in the mature threshed grain from the G area exceeded that from the P area by 3.29 and 2.73 times, respectively, for 1929 and 1931.

The above values indicate that more nitrogen was available in the soil of the spot than in the adjacent soil. The quantitative determination of the nitrate nitrogen actually present in the soil gave additional information as to available nitrogen, at least at the time the samples were taken. These data show that even though four and a half times as much nitrogen had been removed from the G soil by the growing plants as from the P soil, there was still present in the G soil, for the three years under study, averages of 2.59, 3.47, and 7.32 times as much NO_3 as was present in the P soil. Furthermore, of the 121 comparisons, there were only 10 in which the P sample contained more NO_3 than the G sample, and the difference in favor of the P sample was significant in only two instances. (Table 7.)

The relatively low NO_3 content of the P soil, averaging only 6.5 p. p. m. for the 43 samples collected in 1931, together with the low nitrogen content of plant material and the absence of dark-green color, indicated that the plants growing therein were maintaining the nitrate level very near the minimum at which absorption can take place, whereas in most of the G soils there was a surplus of available nitrogen.

These data show beyond doubt that more nitrogen was being rendered available for plant metabolism in the soil of the spots than in the soil immediately surrounding them. The next question that arises is, What factor or factors are responsible for this apparently abnormal condition? Seemingly, any one or any combination of three possible conditions might result in the formation of more available nitrogen in the soil of the spots: (1) The microflora of the spots might be more efficient in transforming nonavailable nitrogen into an available form; (2) the environmental conditions of the microflora of the spots under field conditions might be more favorable; or (3) there might be present in the soil of the spots more nitrogen capable of being readily transformed into an available form.

Almost conclusive evidence that the efficiency of the microflora of the two samples of soil were not significantly different is furnished by the 1931 data secured from the samples to which cottonseed meal was added and which were subsequently incubated. In the presence of an excess of organic nitrogen capable of being readily transformed into the nitrate condition the soil with the most active or efficient microflora should accumulate NO_3 most rapidly. Under such conditions it was found that the average transformation of cottonseed-meal nitrogen into nitrate nitrogen was at the rate of 1,004.5 and 1,040.9 p. p. m., respectively, for the G and P samples. There are certainly no indications in these data that the microflora of P was in any way less efficient than that of G.

That the greater quantity of available nitrogen in the spots is not primarily due to environmental conditions is obvious from the fact that in samples of P and G soil incubated under identical conditions of moisture, temperature, aeration, etc., the differences in nitrate-nitrogen content in favor of the G soil were not only maintained but in many instances were actually accentuated (the initial NO_3 content of G being 2.59 times that of P in the 1929 series, while the NO_3 formed in G during six weeks' incubation was 3.39 times that formed in P).

This leaves as the only plausible explanation a difference in the nitrogen content of the soils, either quantitative or qualitative. The quantitative total nitrogen analyses of the soil lend considerable support to this explanation. Of the 121 comparisons between the nitrogen content of P and G, the latter contained a higher percentage of nitrogen in 104 instances, the average difference in favor of G being 0.0145, 0.0107, and 0.0108 per cent, respectively, for the three years. Evidence that the difference in the relative ability of the two samples to accumulate NO_3 is associated with this difference in total nitrogen content is presented in Table 5. However, the low correlation coefficient for 1929, together with the uniformly low NO_3 accumulation in the P and high accumulation in the G samples regardless of the total nitrogen content, as well as the absence of a direct quantitative relationship between the excess total nitrogen and increased NO_3

accumulation (see next to last paragraph, p.141), points conclusively to a qualitative as well as a quantitative difference in the nitrogen content of the soils.

Field observations have led to the conclusion that the greater number, though not all, of the sort of spots under study follow the deposition of urine by grazing cattle. Cow urine, of course, contains nitrogen in the form of urea which can be readily transformed into nitrate nitrogen. If the deductions made in the preceding paragraphs are correct, it should be possible to duplicate these results experimentally. Experiments were carried out in 1929–30 and 1930–31 to see if this were possible. Certain of the data obtained are presented in Tables 8 and 9. The plots were 10 by 20 feet in size. The yields on the check plots for 1930–31 indicate that nitrogen, at least when applied alone, was not a limiting factor in the soil on which the plots for that year were located. It should be noted, however, that the applications of nitrogen, particularly urine, resulted in the production of plots to all appearances similar to spots, but maturity was slightly delayed and such plots were much more seriously injured by a severe late drought. From these experiments it is evident that the essential characteristics of typical naturally occurring spots, i. e., increased growth accompanied by dark-green color, increased NO_3 content of soil, increased yield, and increased protein content of the grain—can be produced experimentally by the application of cow urine and some other forms of nitrogen.

TABLE 8.—*Data from experimentally produced spots, 1930*

Treatment	Height of plants	Weight of plants	Nitrogen in plant material	NO_3 in soil
	Inches	*Grams*	*Per cent*	*P. p. m.*
Check	14.0	19.6	1.440	12.39
$Ca(CN)_2$	20.0	34.1	1.515	9.48
$CO(NH_2)_2$	20.0	40.5	1.560	28.66
Urine	19.0	33.9	2.270	57.76
Check	14.0	16.2	1.540	20.21

TABLE 9.—*Yield and protein content of grain from experimentally produced spots*

Treatment	1929–30		1930–31	
	Yield per acre	Protein in grain	Yield per acre	Protein in grain
	Bushels	*Per cent*	*Bushels*	*Per cent*
Check	[a] 14.5	10.5	22.9	11.65
Urine in August, ⅛ inch	20.5	12.1	18.9	15.05
Urea in August, 110 pounds per acre	19.1	10.7	24.9	14.55
Check	8.9	11.1	23.4	11.85
Cyanamid in August, 220 pounds per acre	15.6	10.2	26.7	13.50
Urine in March, ⅛ inch	18.3	16.6	28.6	15.75
Check	9.5	11.2	26.4	11.35
Urea in March, 110 pounds per acre	19.9	10.4	28.4	13.85
Cyanamid in March, 220 pounds per acre	19.3	9.8	28.5	13.25
Check	7.5	10.9	24.6	11.85
Urea, 55 pounds in August, 55 pounds in March	20.6	9.6	22.0	14.25
Cyanamid, 110 pounds in August, 110 pounds in March	18.5	9.8	23.2	14.00
Check	10.5	10.6	25.5	10.55
Superphosphate (20 per cent), 300 pounds per acre	10.5	10.6	.....	.....
Potassium chloride, equivalent K in urine	11.1	10.4	.....	.....
Urea at seeding, equivalent N in urine	25.6	13.9	.....	.....
Amophos (11–46–0), [b] 100 pounds at seeding	.....	.....	35.6	13.05
Cyanamid, 200 pounds at seeding	.....	.....	26.6	12.05
Cyanamid, 400 pounds at seeding	.....	.....	26.9	14.50

[a] Yield abnormally high, plot adjacent to road and subject to drainage and blowing from road.
[b] Eleven per cent nitrogen, 46 per cent P_2O_5, 0 per cent potassium.

Of major interest from a practical point of view is the effect of such spots on the yield and quality of the grain. Inquiry among farmers as to the yield on such spots elicited a variety of answers, which may be summed up in the statement that ordinarily the spots outyield the field, but in occasional years the grain of the spots does not fill out, and hence the yield is small. The writers' observations bear this out. The only years for which yield data are available for naturally occurring spots are 1929 and 1931, when the yield from the spots was, respectively, 2.89 and 2.12 times that from the adjacent area. Data for experimentally produced spots in 1930 show differences in yield of the same magnitude. (Table 9.) The years in which spots fail to outyield the adjacent areas are probably associated with one of two circumstances: (1) Conditions so favorable for NO_3 formation in soil that the field at large is supplied an adequate quantity of nitrate nitrogen for maximum yield; or (2) conditions of moisture such that the quantity available late in the season is inadequate for the excessive demands of the heavier growth on the spots.

Of almost equal importance with the increased yield is the marked increased protein content of the grain, both from the naturally occurring and from the experimentally produced spots. The effect of this additional available nitrogen upon the composition of the grain is evident even in the 1931 experimental spots, where questionable increases in yield were recorded.

Because lodging of grain frequently occurs on soils that contain excessive quantities of nitrogen, it is commonly believed that excessive nitrogen is deleterious. In many instances no doubt it is, but during the past three years such has not been found to be the case in the type of spot under study. In order to secure concrete evidence as to the effect that the spots produce on the strength of the straw, breaking tests of the 22 samples of straw harvested for yield in 1929 were conducted with the apparatus constructed by Salmon.[5] Ten separate tests, comprising 10 straws per test, were carried out on each sample. With one exception, the average breaking strength of the G samples was greater than that of the P samples, the average resistance to breaking being 37.5 per cent greater in the case of G.

Thus far the writers' studies have been confined entirely to nitrogen for the reason that this investigation is a part of the general nitrogen studies under way at this station. No evidence is as yet available to show that other elements may not have had some part in the production of the spots. However, applications of potassium under central and western Kansas conditions have been without effect upon the yield of wheat. Applications of phosphorus do affect yields, particularly in eastern Kansas. However, the thesis upon which this work was based is that the spots arise primarily from cow urine, which contains only a trace of phosphorus. Furthermore, the phosphorus content of the plants taken from the P and G areas in 11 selected fields was found to average, respectively, 0.283 and 0.269 per cent. Neither phosphorus nor potassium experimentally applied alone, the latter in the same concentration in which it occurs in urine, gave any indication of producing characteristic spots. (Table

[5] SALMON, S. C. AN INSTRUMENT FOR DETERMINING THE BREAKING STRENGTH OF STRAW, AND A PRELIMINARY REPORT ON THE RELATION BETWEEN BREAKING STRENGTH AND LODGING. Jour. Agr. Research 43: 73–82, illus. 1931.

9.) The effect of both these nutritive elements is being studied further.

No effort has been made to correlate the occurrence of the type of spots under study with agricultural practices such as methods and time of seed-bed preparation. However, early and thorough preparation are known to be essential to an accumulation of adequate nitrate nitrogen for good wheat yields. The very frequent observation that spots occur on low, heavy soils potentially rich in nitrogen—i. e., soils containing 0.15 to 0.20 per cent, many of which are poorly prepared—emphasizes the necessity of such preparation of the seed bed as will enable the nitrifying organisms to function properly if maximum yields are to be expected. On the other hand, the data given herein relative to the nitrogen content of Kansas soils (the most comprehensive thus far presented) show that efforts are being made to grow wheat upon many soils that are relatively deficient in nitrogen. It is upon such soils that the most striking spots are observed. Continuous cropping to nonleguminous crops will continue to reduce the nitrogen level until an equilibrium is reached,[6] and its is logical to expect the spotted condition to become more prevalent as time goes on. The data in Table 9, together with much unpublished data, indicate that on many soils the use of commercial nitrogenous fertilizers will, under certain conditions, result in marked increases in yield, the conditions being a low concentration of available nitrogen, unaccompanied by a deficiency in some other nutritive element. The response to such treatment may be expected to increase in the future.

SUMMARY

This paper presents the results of three years' study of the rôle of nitrogen in the production of the type of spot frequently observed in small-grain fields in Kansas. These spots are almost invariably accompanied by a higher total nitrogen content of the soil, higher NO_3 content of the soil, higher percentage nitrogen composition of the growing plant, higher total nitrogen absorption, higher yield of grain, and higher protein content of the grain. The increased total and available nitrogen in the soil of the spot does not appear to be associated with a more active nitrogen-fixing or nitrifying microflora. Spots identical in appearance and in quantitative measurements have been produced experimentally by the application of cow urine and certain other nitrogen-containing materials. All evidence thus far obtained points to the conclusion that the spots are the direct result of the presence of a more abundant supply of available nitrogen in the soil of these limited areas, particularly during the early spring growing period; and that this increased available nitrogen has arisen from a limited quantity of nitrogen, either already available or capable of being readily transformed into an available condition, finding its way into the soil, in most instances through the deposition of urine.

[6] GAINEY, P. L., SEWELL, M. C., and LATSHAW, W. L. THE NITROGEN BALANCE IN CULTIVATED SEMI-ARID WESTERN KANSAS SOILS. Jour. Amer. Soc. Agron. 21: 1130–1153. 1929.

NITROGEN CHANGES PRODUCED IN CERTAIN NITROGENOUS COMPOUNDS BY AZOTOBACTER AND THE NITROGEN FIXED IN THE PRESENCE OF THESE COMPOUNDS[1]

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INTRODUCTION

Soils contain varying amounts of organic nitrogen, mostly in the form of complex organic compounds, but the amount of mineral nitrogen present is usually small. It is therefore believed that most soils do not contain sufficient nitrogen, either organic or mineral, to depress nitrogen fixation by Azotobacter to any large extent. As some of these complex nitrogenous compounds are stimulative to fixation by Azotobacter, a soil rich in organic matter may be more stimulative to nitrogen fixation than a poor soil.

To determine the effect of various nitrogenous compounds on nitrogen fixation, most investigators have measured the amount of nitrogen fixed in the presence of these compounds, but there has been comparatively little study of the nitrogen changes produced or the amounts of these compounds utilized. In most of this work it has been found that the simple nitrogenous compounds have a greater depressing effect on nitrogen fixation by Azotobacter than the more complex amino acids and proteins. This seems to indicate that the simple nitrogenous compounds may be utilized to a much greater extent than the more complex substances, and hence, in the latter case, the organisms must use the atmospheric nitrogen in order to make a vigorous growth. There is also the possibility that the more complex compounds are more stimulative to growth and hence bring about a greater fixation.

The purpose of the work reported here was to determine the utilization and the nitrogen changes produced by Azotobacter in various nitrogenous compounds, and the effect of these compounds upon the growth and nitrogen fixation of several species of Azotobacter.

REVIEW OF LITERATURE

Numerous investigations have been carried out to determine the effect of nitrogen compounds on the growth of Azotobacter and the fixation of nitrogen.

Hills (*10*)[3] found that the nitrates of potassium, sodium, and calcium increased the growth of Azotobacter, and also the fixation of nitrogen when the amount added was not more than 100 mg per 100 c c of culture medium.

[1] Received for publication Jan. 19, 1932; issued August, 1932. Journal Paper No. B 34 of the Iowa Agricultural Experiment Station. Part of a thesis submitted to the graduate faculty of Iowa State College in partial fulfillment of the requirements for the degree of doctor of philosophy.

[2] The writer wishes to thank Dr. P. E. Brown for his helpful suggestions in planning the experimental work and in the preparation of the manuscript, and Dr. F. B. Smith for the help which he has given during the progress of the work.

[3] Reference is made by number (italic) to Literature Cited, p. 160.

Kostytschew, Ryskaltschuk, and Schwezowa (*12*) found that the addition of 15 mg of NH_3 nitrogen, 24 mg of $NaNO_3$ nitrogen, or 130 mg of peptone nitrogen to 100 c c of culture medium practically inhibited nitrogen fixation by *Azotobacter agile*. They also found small amounts of ammonia in young cultures of this organism and concluded that this was probably the first product of nitrogen fixation.

Lipman (*13*) noted that 50 mg of potassium nitrate, or 100 mg of peptone per 100 c c of solution depressed nitrogen fixation by *Aztobacter vinelandii*.

Reed and Williams (*16*) studied the effect of various organic compounds, including many which are toxic to higher plants, on the growth of Azotobacter and found that nitrogen fixation was only slightly influenced by most of the compounds used. In concentrations that were fatal to certain higher plants, many of the compounds depressed fixation only slightly. Certain of the compounds, especially urea, glycocoll, formamide, and allantoin at concentrations of 500 parts per million were particularly active in depressing nitrogen fixation. It was suggested that this was not due to toxic effects but rather to the fact that the compounds were utilized by Azotobacter.

Winters (*19*) observed that rapid nitrogen fixation in the soil was at first accompanied by a decrease in nitrate content, which was probably due to the assimilation of nitrates by the organisms. Applications of 100 pounds of sodium nitrate per acre or an equivalent amount of nitrogen as ammonium sulphate or calcium nitrate stimulated nitrogen fixation.

Zoond (*20*) found that increasing concentrations over 3 mg of nitrate, amino acid, or peptone decreased the amount of nitrogen fixed by Azotobacter. Sterile, unheated plant extracts increased nitrogen fixation when applied in moderate amounts.

Löhnis and Green (*14*) found that manure, straw, and peat stimulated nitrogen fixation when used in a synthetic culture medium.

Murray (*15*) found that 1 per cent of plant residues when added to soil, greatly stimulated nitrogen fixation. When the same plant materials were added to sterile sand inoculated with Azotobacter only a slight stimulation was observed.

Brown and Allison (*2*), after studying the effect of manure and various plant residues, found that the nitrogen-carbon ratios of the materials used were of little or no value in indicating their effects on nitrogen fixation.

Greaves (*7*) found that soils comparatively rich in combined nitrogen fixed more nitrogen than did those poorer in organic nitrogen.

Burk and Lineweaver (*3*) found that the quantity of readily available fixed nitrogen required to inhibit nitrogen fixation by Azotobacter was 0.5 mg per 100 c c of solution.

Fuller and Rettger (*6*) tested the effects of a large number of nitrogen compounds on nitrogen fixation by Azotobacter and noted that most of the nontoxic compounds did not influence nitrogen fixation to any great extent. Certain of the compounds such as asparic acid, cysteine hydrochloride, glycocoll, creatine, creatinine, and urea practically inhibited nitrogen fixation.

The action of Azotobacter upon nitrogen-containing compounds has been studied to some extent.

Beijerinck and Van Delden (*1*) found that Azotobacter reduced nitrites directly to ammonia.

Stoklasa et al. (*17*) found that nitrates were reduced to nitrites and then to ammonia, which was then utilized probably for the building of amino acids and proteins. These findings were confirmed by Kostytschew, Ryskaltschuk, and Schwezowa (*12*).

Waynick and Woodhouse, as reported by Hunt (*11*), observed that appreciable amounts of amino nitrogen were present in young cultures of Azotobacter.

Halversen (*8*) found that the amounts of amino nitrogen increased with the age of the culture up to a certain point and then decreased. Nitrites and nitrates could not be detected at any time in vigorously growing cultures, provided the reagents used in making up the solutions were free from these compounds. As ammonia was always found in cultures during the first few days of growth, it was concluded that ammonia was probably the first product of nitrogen fixation.

Fuller and Rettger (*6*) found that peptone, nucleic acid, aspartic acid, and glycocoll were readily utilized by Azotobacter. Tryptophane and glutamic acid were partly utilized, while tryosine was only slightly utilized.

METHODS

The medium used in these studies was nitrogen-free mannitol solution prepared according to the directions of Fred and Waksman (*5*). The composition of the medium was as follows:

Mannitol	10 g	Manganese sulphate	Trace
Di-potassium phosphate	0.5 g	Ferric chloride	Trace
Magnesium sulphate	.2 g	Distilled water	1,000 c c
Sodium chloride	.2 g		

The nitrogenous substance to be tested was added at the rate of 1 g per liter of this medium. The reaction of the medium was adjusted to pH 7.4 by adding a 1 per cent solution of sodium hydroxide, and using brom thymol blue as the indicator. One hundred c c of this medium was added to each of ten 500 c c Erlenmeyer flasks, which were then sterilized and inoculated in duplicate with each of the following organisms: *Azotobacter chroococcum*, *A. beijerinckii*, *A. vinelandii*[4] and Azotobacter No. 2. The flasks were incubated at 26° to 28° C. for three weeks, then transfers were made to peptone mannitol agar slants to test the purity of the cultures. Any common contaminating organisms made a very good growth on this medium and could be detected readily. The various forms of nitrogen present in the cultures were determined as quickly as possible at the end of the incubation period.

Alpha amino nitrogen was determined on 6 to 10 c c aliquots with the Van Slyke macro apparatus (*18*). To increase the accuracy of the apparatus, the macro-gas burette was replaced by a micro-gas burette.

Ammonia nitrogen was determined on 25 c c aliquots by the aeration method. One gram of anhydrous sodium carbonate was added to 25 c c of the culture solution in aeration tubes, which were attached to similar tubes containing a known quantity of standard acid, and the aeration was continued for five hours. The acid was then titrated with standard alkali.

[4] These species were secured through the courtesy of N. R. Smith, Bureau of Chemistry and Soils, U. S. Department of Agriculture.

Nitrites were determined by the alpha-naphthylamine sulphanilic acid method according to Fred and Waksman (*5*).

Nitrates were determined by the phenoldisulphonic acid method as modified by Harper (*9*).

Total nitrogen was determined on 25 c c aliquots by the Kjeldahl method. Copper sulphate was used as a catalyst in digestion instead of mercury. When nitrates or nitrites were present in the solutions, they were reduced to ammonia according to the method of Davisson and Parsons (*4*). Then the digestion and the distillation were carried out in the usual way.

Unless it is stated otherwise, all solutions were incubated for three weeks. The difference in the amount of amino nitrogen in the uninoculated and in the inoculated solutions is given in the tables as the amount of amino nitrogen utilized.

RESULTS

The results in Table 1 show the nitrogen changes produced after three weeks incubation in a solution containing 1 g of KNO_3 per liter. These results show that a large part of the nitrate nitrogen was utilized by the organisms. Only *Azotobacter vinelandii* and Azotobacter No. 2 fixed any measurable amount of nitrogen in the presence of this quantity of nitrate. There was a small amount of amino acid nitrogen present in the solutions, indicating that the nitrate probably passed through this form as it was utilized by the organisms.

TABLE 1.—*Nitrogen changes produced by various organisms after three weeks incubation in mannitol media containing different chemicals*

[Results are expressed as milligrams of nitrogen per 100 c c of solution for the averages of duplicate flasks]

MEDIUM CONTAINING 1 G OF POTASSIUM NITRATE PER LITER

Organism	Amino nitrogen	Ammonia	Nitrites	Nitrates	Total nitrogen
Check	(a)	(a)	(a)	13.7	13.8
Azotobacter chroococcum	1.35	(a)	(a)	3.86	13.8
A. beijerinckii	1.42	(a)	(b)	6.17	13.8
A. vinelandii	2.10	(a)	(a)	3.53	15.5
Azotobacter No. 2	2.10	(a)	(a)	(a)	14.6

MEDIUM CONTAINING 1 G OF AMMONIUM SULPHATE PER LITER

Check	3.8	21.4	(a)	(a)	21.2
Azotobacter chroococcum	4.03	18.5	(a)	(a)	20.8
A. beijerinckii	4.03	19.1	(a)	(a)	20.4
A. vinelandii	4.74	18.8	(a)	(a)	22.6
Azotobacter No. 2	3.9	19.1	(a)	(a)	20.4

MEDIUM CONTAINING 1 G OF UREA PER LITER

Check		3.38	(a)	(a)	47.2
Azotobacter chroococcum		17.6	(a)	(a)	38.8
A. beijerinckii		12.4	(a)	(a)	39.6
A. vinelandii		24.4	(a)	(a)	34.8
Azotobacter No. 2		10.1	(a)	(a)	48.4

[a] None. [b] Trace.

As there was a trace of nitrite in the culture of *Azotobacter beijerinckii*, it was decided to test for nitrites and ammonia after short periods of incubation. Sodium nitrate was used in this experiment as it seemed to give somewhat better growth and might therefore permit of the accumulation of nitrites and ammonia.

TABLE 2.—*Nitrogen changes produced by various organisms in a mannitol medium containing 1 g of $NaNO_3$ per liter after various periods of incubation*

[Results expressed as milligrams of nitrogen per 100 c c of solution for averages of duplicate flasks]

Organism	Nitrogen in form indicated produced after incubation of—									
	4 days			6 days			8 days			14 days
	Nitrates	Nitrites	Ammonia	Nitrates	Nitrites	Ammonia	Nitrates	Nitrites	Ammonia	Total nitrogen
Check	13.7	(a)	(a)	13.8	(a)	(a)	13.6	(a)	(a)	14.02
Azotobacter chroococcum	12.3	(b)	(b)	8.1	(b)	(b)	6.2	(a)	(b)	14.07
A. beijerinckii	12.9	0.02	0.28	9.9	0.02	0.28	7.8	(b)	0.14	13.94
A. vinelandii	12.4	(b)	.56	8.2	.36	1.4	6.4	(a)	.14	14.12
Azotobacter No. 2	10.4	(b)	(b)	7.1	.71	.56	2.3	0.02	.42	14.10

[a] None. [b] Trace.

The results in Table 2 show the changes produced in a solution containing 1 g of $NaNO_3$ per liter. There was a measurable accumulation of nitrites and ammonia in the cultures, which indicates that the nitrates were reduced to nitrites and then to ammonia. Probably the reason there was not a larger accumulation of nitrites and ammonia was because of a rapid utilization. The total nitrogen determinations at the end of 14 days show no loss of nitrogen indicating that all the nitrates were utilized by the bacteria, or, if there was a loss, the difference was made up by nitrogen fixation. It would seem more reasonable to believe that the nitrates were used in preference to free nitrogen and that there was no fixation of free nitrogen during the reduction process. In this experiment Azotobacter No. 2 did not utilize all the nitrate nitrogen. In some other experiments where the growth was somewhat better, all the nitrate was utilized in about seven to eight days in the case of this organism.

The different species of Azotobacter were grown in methylene blue whole milk, and it was found that the organisms which reduced nitrates to the greatest extent also reduced methylene blue in a shorter period of time.

A test was also made to determine whether they would grow on nitrate agar in sealed tubes. No growth occurred, while there was excellent growth on the check tubes, which were not sealed. Probably these organisms reduce nitrates in order to secure the nitrogen and not the oxygen.

Nitrites added to a mannitol solution at the rate of 10 mg per liter were practically all reduced in two days, and after three days they were all reduced by these four species of Azotobacter.

Table 1 shows the nitrogen changes produced in a solution containing 1 g of ammonium sulphate per liter. There was no fixation of nitrogen except possibly in the case of *Azotobacter vinelandii*, but there

was a slight loss of nitrogen probably as ammonia. While the figures for amino nitrogen include considerable ammonia nitrogen, they are at least comparable. The Van Slyke method gives results in the presence of ammoniacal nitrogen which are comparable if the time of making all the determinations is the same. The ammonia reacts slowly so that if three minutes are used for making the determinations, about 20 per cent of the ammonia is included in the amino nitrogen determinations. As all the determinations reported here were made in three minutes, the results are at least comparable. All the cultures contained less ammonia nitrogen than did the check, but more amino nitrogen, which indicates that there was a measurable amount of amino nitrogen present, especially in the cultures of *A. vinelandii*.

As no nitrates or nitrites could be detected, the ammonia must have been used by the organisms. The growth was not very good, which indicates that the sulphuric acid remaining after the utilization of the ammonia, made the medium more acid than was favorable for the growth of the organisms.

In the solution containing 1 g of urea per liter, a large amount of ammonia was produced which shows that all the organisms were very active in decomposing urea. Tests of these cultures would probably show the presence of a large quantity of the enzyme urease, which is active in changing urea to ammonia. *Azotobacter vinelandii* was especially active in changing urea to ammonia, while *A. chroococcum* was almost as active. There was a loss of nitrogen from all the cultures except Azotobacter No. 2, and in this culture there was a slight fixation. The quantity of nitrogen fixed was too small, however, to be significant. There was less ammonia produced in this culture than in any of the other cultures, so there probably was no loss of ammonia, or, if there was a loss, it was made up by nitrogen fixation. There is a correlation between the amount of nitrogen lost and the amount of ammonia produced, e. g., the cultures containing the most ammonia contained the least total nitrogen. This shows that the nitrogen was undoubtedly lost in the form of ammonia.

In the Van Slyke method the urea as well as the ammonia reacts slowly with the nitrous acid to give free nitrogen gas, so that the results for amino nitrogen include a part of the ammonia and also a part of the urea nitrogen. For this reason the figures are not given here. It may be stated, however, that the quantity of amino nitrogen, as determined under these conditions, was very much higher in the inoculated than in the uninoculated cultures, which indicates that there may have been considerable amino nitrogen present.

Table 3 shows the nitrogen changes produced in mannitol medium containing 1 g of a-alanine per liter. The only organism that used alanine to any extent was *Azotobacter vinelandii*. Azotobacter No. 2 used a very small amount, but this amount is probably not significant. In the three instances where alanine was not utilized to any extent, it seemed to stimulate nitrogen fixation slightly. Where alanine was utilized, however, there was a depressing effect on nitrogen fixation. The amount of nitrogen fixed, both in the presence and in the absence of alanine, was rather low, probably because there was no calcium in the medium. Tests made after this experiment was completed show that all these organisms will fix considerably more nitrogen in the presence of calcium. There was a small amount of ammonia produced by *A. vinelandii* but this may have been within the

experimental variation. It may also be possible that the alanine was decomposed to ammonia before it was utilized by the organism.

TABLE 3.—*Nitrogen changes produced by various organisms in a mannitol media containing various amino acids and nitrogen compounds*

[Results are expressed as milligrams of nitrogen per 100 c c of solution for the averages of duplicate flasks]

MEDIUM CONTAINING 1 G OF A-ALANINE PER LITER

Organism	Nitrogen fixed	Amino nitrogen	Amino nitrogen utilized	Ammonia	Nitrogen-free medium nitrogen fixed	Total nitrogen
Check		15.9		(a)		16.0
Azotobacter chroococcum	2.6	15.9	0	(a)	1.8	18.6
A. beijerinckii	1.8	16.4	−.5	(a)	1.6	17.8
A. vinelandii	4.0	13.3	2.6	1.56	5.7	20.0
Azotobacter No. 2	5.4	15.5	.4	(a)	3.4	21.4

MEDIUM CONTAINING 1 G OF DL-VALINE PER LITER

Check		15.4		(a)		15.6
Azotobacter chroococcum	0.6	14.2	1.2	(a)	1.8	16.2
A. beijerinckii	4.3	14.7	.7	(a)	1.6	19.9
A. vinelandii	.1	13.6	1.8	(a)	5.7	15.7
Azotobacter No. 2	.9	13.8	1.6	(a)	3.4	16.5

MEDIUM CONTAINING 1 G OF DL-A-AMINO-N-BUTYRIC ACID PER LITER

Check		14.5		(a)		14.6
Azotobacter chroococcum	0.6	14.8	−0.3	(a)	1.8	15.2
A. beijerinckii	3.9	14.8	−.3	(a)	1.6	18.5
A. vinelandii	.9	12.3	2.2	0.56	5.7	15.5
Azotobacter No. 2	2.2	14.5	0	(a)	3.4	17.8

MEDIUM CONTAINING 1 G OF ASPARAGIN PER LITER

Check		11.1		(a)		20.5
Azotobacter chroococcum	1.3	11.5	−0.4	5.32	1.8	21.8
A. beijerinckii	.4	11.6	−.5	5.04	1.6	20.9
A. vinelandii	.9	7.7	3.4	6.16	5.7	21.4
Azotobacter No. 2	.7	10.8	.3	3.92	3.4	21.2

MEDIUM CONTAINING 2 G OF EGG ALBUMIN PER LITER

Check		(a)	(a)	(a)		24.8
Azotobacter chroococcum	3.8	(a)	(a)	(a)	1.8	28.6
A. beijerinckii	3.8	(a)	(a)	(a)	1.6	28.6
A. vinelandii	3.0	(a)	(a)	(a)	5.7	27.8
Azotobacter No. 2	5.3	(a)	(a)	(a)	3.4	30.1

MEDIUM CONTAINING 2 G OF PEPTONE PER LITER

Check		8.17		(a)		34.8
Azotobacter chroococcum	2.7	7.33	0.84	(a)	1.8	37.5
A. beijerinckii	2.9	8.09	.08	(a)	1.4	37.7
A. vinelandii	3.0	5.57	2.60	0.56	5.7	37.8
Azotobacter No. 2	2.6	7.49	.68	(a)	3.4	37.4

MEDIUM CONTAINING 2 G OF CASEIN PER LITER

Check		3.65		(a)		34.5
Azotobacter chroococcum	1.6	3.49	0.16	(a)	1.8	36.1
A. beijerinckii	.9	2.72	.93	(a)	1.6	35.6
A. vinelandii	2.5	2.31	1.34	(a)	5.7	37.0
Azotobacter No. 2	2.7	3.68	−.03	(a)	3.4	37.2

[a] None.

TABLE 3.—*Nitrogen changes produced by various organisms in a mannitol media containing various amino acids and nitrogen compounds*—Continued

MEDIUM CONTAINING 1 G OF L-TYROSINE PER LITER

Organism	Nitrogen fixed	Amino nitrogen	Amino nitrogen utilized	Ammonia	Nitrogen-free medium nitrogen fixed	Total nitrogen
Check		9.1		(a)		8.9
Azotobacter chroococcum	2.2	9.3	−0.2	(a)	2.5	11.1
A. beijerinckii	1.9	9.2	−.1	(a)	2.2	10.8
A. vinelandii	10.1	7.9	1.2	(a)	8.6	19.0
Azotobacter No. 2	3.1	9.0	.1	(a)	5.9	12.0

MEDIUM CONTAINING 1 G OF D-GLUTAMIC ACID PER LITER

Check		9.80		(a)		9.69
Azotobacter chroococcum	5.20	9.52	0.28	(a)	2.5	14.89
A. beijerinckii	.56	9.91	−.11	(a)	2.2	10.25
A. vinelandii	2.80	5.94	3.86	(a)	8.6	12.49
Azotobacter No. 2	(a)	6.32	3.48	(a)	5.9	9.69

MEDIUM CONTAINING 1 G OF DL-A-AMINO-N-VALERIC ACID PER LITER

Check		12.6		(a)		12.40
Azotobacter chroococcum	2.07	12.7	−0.1	(a)	2.5	14.47
A. beijerinckii	1.68	12.9	−.3	(a)	2.2	14.08
A. vinelandii	3.36	10.5	2.1	(a)	8.6	15.76
Azotobacter No. 2	3.20	11.2	1.4	(a)	5.9	15.60

MEDIUM CONTAINING 1 G OF DL-PHENYLAIANINE PER LITER

Check		8.70		(a)		8.64
Azotobacter chroococcum	3.08	7.80	0.90	(a)	2.5	11.72
A. beijerinckii	2.10	8.60	.10	(a)	2.2	10.74
A. vinelandii	8.70	7.70	1.00	(a)	8.6	17.34
Azotobacter No. 2	5.74	7.75	.95	(a)	5.9	14.38

[a] None.

Glycocoll at a concentration of 1 g per liter was toxic to the organisms, and very poor growth was secured. The amounts of amino nitrogen utilized or the amounts of nitrogen fixed were very small, so the figures are not given here. At lower concentrations glycocoll probably would not be toxic and might be utilized by the organisms.

The results in Table 3 show that where the utilization of dl-valine was greatest, the amount of nitrogen fixed was the smallest. The very small amount of the amino nitrogen used by *Azotobacter beijerinckii* was probably not significant. This seems to indicate that *A. beijerinckii* could not use the valine and hence had to secure its nitrogen from the atmosphere. The valine seemed to be very stimulative to its growth. In the case of the other organisms, however, the valine depressed nitrogen fixation, probably not because it was toxic, but because it was utilized in preference to free nitrogen.

A mannitol medium containing 1 g of dl-a-amino-n-butyric acid per liter depressed nitrogen fixation by all the organisms except *Azotobacter beijerinckii* in which it stimulated fixation. This seems to indicate that it must have been toxic at this concentration to all the organisms except *A. beijerinckii*. In the case of *A. vinelandii*, however, the

depressed fixation may have been due to the utilization of the amino acid in preference to free nitrogen. It seems that *A. beijerinckii* can withstand a higher concentration of this amino acid without a toxic effect than is true of the other organisms. Many compounds that are toxic at higher concentrations are stimulative at lower concentrations. The results seem to indicate that this may be the case with many of the amino acids, especially if they are not utilized.

The results show that asparagin was utilized only by *Azotobacter vinelandii* and Azotobacter No. 2. In the inoculated solutions, the quantities of amino nitrogen were higher than they should be because part of the ammonia nitrogen was included in the determinations. As 6 c c aliquots were used in making the amino acid determinations, the quantity of ammonia present would be less than 1 mg, and 21 per cent of this amount would be about 0.2 mg (the percentage of ammonia nitrogen when the time of making the determinations is three minutes). As each of these determinations was made in three minutes, the amount of ammonia included would be small, e. g., not more than 0.3 or 0.4 mg. The extra amount of amino nitrogen present in the cultures of *A. chroococcum* and *A. beijerinckii* over that of the check was about equal to the amount of ammonia included in the determinations. It is, therefore, believed that there was no utilization of amino nitrogen in the case of these two organisms.

The amide nitrogen was readily attacked by all the organisms, with the production of large amounts of ammonia, which was then utilized by the organisms in preference to free nitrogen. This indicates that the amide nitrogen was very effective in depressing fixation. Tests were not made with asparic acid, but in all probability all the organisms except *Azotobacter vinelandii* would have fixed considerable amounts of nitrogen.

The egg albumin stimulated nitrogen fixation by all the organisms except *Azotobacter vinelandii* in which it depressed fixation. This seems to indicate that the egg albumin could not be utilized by any of the organisms except *A. vinelandii*. This organism may have used the protein in preference to fixing nitrogen, or the protein may have been slightly toxic. As *A. vinelandii* used practically every amino acid tested, there is good reason to believe that it used the egg albumin also. No amino acid could be detected in any of the cultures when the Van Slyke method was used to make the determinations. This indicates that the organisms were unable to break down the egg albumin into amino acids, or, if they did, the amino acids were all utilized by the end of the three weeks' period. Ammonia was not detected in any of the cultures.

The peptone stimulated fixation by all the organisms except *Azotobacter vinelandii* and Azotobacter No. 2. The depressed fixation in these cases was probably due to the fact that these organisms used the amino acids in preference to the free nitrogen. *A. chroococcum* and Azotobacter No. 2 utilized a small amount of amino nitrogen, but *A. beijerinckii* used practically none. The presence of the peptone, however, seemed to stimulate this organism to fix much more nitrogen. There was a small amount of ammonia produced by *A. vinelandii*, but this amount may not be significant. There was also the possibility that the amino acids were decomposed to ammonia and then utilized by *A. vinelandii*.

In a mannitol medium containing 2 g of casein per liter, nitrogen fixation was depressed in all cases, but it was depressed to a greater extent in the case of *Azotobacter vinelandii* and *A. beijerinckii*. This was probably due to the fact that these two organisms used the amino nitrogen instead of free nitrogen. *A. chroococcum* and Azotobacter No. 2 utilized practically none of the amino nitrogen, and fixation was only very slightly depressed.

Because of the low nitrogen fixation secured in the previous work, it was decided to use calcium carbonate in the following experiments. The same medium was used, except that 3 g of $CaCO_3$ per liter was added.

L-tyrosine was not utilized by any of the organisms except *Azotobacter vinelandii*, but it seemed to have a depressing effect on nitrogen fixation by all the organisms except *A. vinelandii*, in which there was a slight stimulation. Although the amino acid was utilized by *A. vinelandii*, it did not depress fixation. This may be due to the phenol group, which seemed to stimulate this organism to a greater growth but was slightly toxic to the other organisms. Probably at a higher concentration this substance would have been toxic to *A. vinelandii*, whereas at a lower concentration it may have stimulated the other organisms.

All the organisms utilized d-glutamic acid, except *Azotobacter beijerinckii*, but the amount used by *A. chroococcum* was very small. *A. chroococcum* fixed a large amount of nitrogen, which shows that the amino acid was stimulative to this organism. The amino acid was undoubtedly toxic to *A. beijerinckii*, however, as it made a very poor growth. In the case of *A. vinelandii* and Azotobacter No. 2, nitrogen fixation was greatly depressed, probably because the amino acid was utilized in preference to free nitrogen.

A mannitol medium containing 1 g of dl-a-amino-n-valeric acid per liter depressed nitrogen fixation only slightly in the case of *Azotobacter chroococcum* and *A. beijerinckii*, but it was very effective in depressing nitrogen fixation in the other two organisms. This was probably due to the fact that *A. chroococcum* and *A. beijerinckii* were unable to use the amino nitrogen and hence had to depend on the process of fixation to secure their nitrogen, whereas the other two organisms used the amino nitrogen as well as the free nitrogen.

The amounts of dl-phenylalanine utilized were very small, except in the case of *Azotobacter vinelandii*, in which there was considerable utilization. The amounts used in the cultures of *A. beijerinckii* were not significant. The phenylalanine did not depress nitrogen fixation, and in the case of *A. chroococcum* there was a slight stimulation. Ammonia was not produced by any of the organisms. The results seem to show that the amino acid was very stimulative to the growth of all the organisms except *A. beijerinckii*, and that in making a vigorous growth the organisms used a large amount of free nitrogen, for they were unable to use any considerable amount of the fixed nitrogen.

Table 4 shows the amounts of nitrogen fixed in a nitrogen-free mannitol medium with and without 1 g of l-cystine per liter. The amounts of nitrogen fixed were slightly higher in all the cultures containing l-cystine than in the nitrogen-free medium. Determinations of amino nitrogen were not made, but it is thought that there was little or no utilization of the cystine for it was insoluble in the medium.

TABLE 4.—*Nitrogen fixed by various organisms in a nitrogen-free mannitol medium and in the same medium containing 1 g of l-cystine per liter*

[Results expressed as milligrams of nitrogen per 100 c c of medium for the averages of duplicate flasks]

Organism	Medium +cystine	Medium alone
Azotobacter chroococcum	2.66	2.5
A. beijerinckii	2.52	2.2
A. vinelandii	8.96	8.6
Azotobacter No. 2	6.00	5.9

DISCUSSION

The results as a whole show that the simple nitrogenous compounds have a greater depressing effect on nitrogen fixation than do the more complex ones due to the fact that the simple compounds are readily utilized by the organisms, whereas many of the complex compounds are only slightly utilized, or in most cases not utilized at all. When the complex compounds depressed fixation, it was generally found that they were either utilized by the organisms or that they were toxic to growth. The amino acids contained in peptone were not utilized to any large extent, except by *Azotobacter vinelandii*, but they stimulated nitrogen fixation in some cases. Certain of the other compounds brought about a stimulation in the fixation by some of the organisms. D-glutamic acid was found to stimulate *A. chroococcum* to a large extent, but it depressed the fixation by all the other organisms. Practically all the compounds tested were utilized to some extent by *A. vinelandii*, and as a result nitrogen fixation was depressed to a greater or less extent. Although l-tyrosine and dl-phenylalanine were found to be utilized by *A. vinelandii*, nitrogen fixation was not depressed. As these were the only instances in which the compounds were utilized and at the same time did not depress nitrogen fixation, it is doubtful whether they were utilized in preference to the free nitrogen. The organism used only about one-eighth of the amino nitrogen present but at the same time fixed from 9 to 10 mg of nitrogen. This seems to show that these two compounds were very stimulative to growth, and that the organism, being unable to use any considerable amount of the amino nitrogen, utilized the free nitrogen for its growth.

Certain of the amino acids, such as d-glutamic acid, dl-a-amino-n-valeric acid, and dl-valine, were utilized in part by Azotobacter No. 2. *Azotobacter chroococcum* was able to utilize some of the amino nitrogen in the compounds such as dl-phenylalanine, dl-valine, and peptone, while *A. beijerinckii* used some of the amino nitrogen of valine and casein. Of the amino acids tested, however, the number as well as the amount utilized by the organisms except *A. vinelandii* was exceedingly small. *A. vinelandii* used practically all of the amino acids tested, but the amounts used were rather small.

The nitrates, nitrites, urea, and the amide nitrogen of asparagin were readily attacked with the production of ammonia which was then utilized by the organisms. A small amount of ammonia was found in the cultures containing certain amino acids, which indicates that certain of the amino acids may be decomposed to ammonia before they are utilized by the organisms. Other amino acids may be of such a structure that they can be utilized directly.

With very few exceptions, all the compounds that were readily utilized inhibited nitrogen fixation entirely. Compounds that were utilized with difficulty depressed nitrogen fixation usually to the extent that they were utilized. In a few cases these latter compounds depressed fixation to a greater extent, and in others they stimulated fixation to a greater or less degree. When the compounds were not utilized, they had little or no effect or they were stimulative to fixation. In a few instances the compounds were toxic at the concentration used and hence greatly depressed growth and nitrogen fixation.

Nitrates usually stimulated pigment formation, but it was possible to secure a good growth in a nitrate solution without pigment formation. In fact, growth seemed to be better in cultures where there was no pigment formation. After pigment was formed, even in a nitrate solution, the growth seemed to be slower, indicating some unfavorable conditions.

SUMMARY

The nitrogen changes produced by four species of Azotobacter in mannitol media containing some 17 different nitrogenous compounds were studied. The amounts of these compounds utilized by the organisms as well as their effect on nitrogen fixation were determined.

The nitrates of sodium and potassium, potassium nitrite, urea, and the amide nitrogen of asparagin were readily attacked with the production of ammonia, which was then utilized by the organisms. These compounds inhibited nitrogen fixation almost entirely, and in some cases there was a loss of ammonia.

All the organisms tested, except *Azotobacter vinelandii*, utilized only a few of the amino acids, and where there was utilization the amount used was very small. *A. vinelandii* utilized practically every amino acid tested, but the amount used was not very large. Where the amino acids were utilized, nitrogen fixation was usually depressed. If the amino acids were not utilized, nitrogen fixation was generally either stimulated or affected not at all. The simple nitrogenous compounds were more readily utilized than the complex compounds.

With the exception of *Azotobacter vinelandii* the organisms that fixed the most nitrogen when grown in a nitrogen-free medium, used the largest amount of nitrate nitrogen when grown in a medium containing nitrates. *A. vinelandii* fixed a large amount of nitrogen when grown on nitrogen-free medium but did not utilize quite all of the nitrates when grown in a medium rich in nitrate nitrogen. In the latter medium it fixed a small amount of nitrogen.

Azotobacter No. 2 was able to utilize 14 mg of nitrate nitrogen per 100 c c of medium in seven to nine days.

Many of the complex compounds were not utilized except to a small extent by *Azotobacter vinelandii*, and nitrogen fixation was usually not affected or was stimulated.

LITERATURE CITED

(1) Beijerinck, M. W., and Delden, A. van
1902. Über die assimilation des freien stickstoffs durch bakterien. Centbl. Bakt. [etc.] (II) 9:3–43.

(2) Brown, P. E., and Allison, F. E.
1916. The influence of some humus-forming materials of narrow and of wide nitrogen-carbon ratio on bacterial activities. Soil Sci. 1:49–75.

(3) Burk, D., and Lineweaver, H.
1930. The influence of fixed nitrogen on azotobacter. Jour. Bact. 19:389–414, illus.
(4) Davisson, B. S., and Parsons, J. T.
1919. The determination of total nitrogen including nitric nitrogen. Jour. Indus. and Engin. Chem. 11:306–311, illus.
(5) Fred, E. B., and Waksman, S. A.
1928. Laboratory manual of general microbiology, with special reference to the microorganisms of the soil. 145 p., illus. New York.
(6) Fuller J. E., and Rettger, L. F.
1931. The influence of combined nitrogen on growth and nitrogen fixation by azotobacter. Soil Sci. 31:219–234.
(7) Greaves, J. E.
1914. A study of the bacterial activities of virgin and cultivated soils. Centbl. Bakt. [etc.] (II) 41:444–459.
(8) Halversen, W. V.
1927. The nitrogen metabolism of nitrogen-fixing bacteria. Iowa State Col. Jour. Sci. 1:395–410.
(9) Harper, H. J.
1924. The accurate determination of nitrates in soils phenoldisulfonic acid method. Jour. Indus. and Engin. Chem. 16: 180–183.
(10) Hills, T. L.
1918. Influence of nitrates on nitrogen-assimilating bacteria. Jour. Agr. Research 12:183–230.
(11) Hunt, T. F.
1919. By what steps does azotobacter fix nitrogen? Calif. Agr. Expt. Sta. Rpt. 1918–19:62–63.
(12) Kostytschew, S., Ryskaltschuk, A., and Schwezowa, O.
1926. Biochemische untersuchungen über azotobacter agile. Hoppe-Seyler's Ztschr. Physiol. Chem. 154:1–17.
(13) Lipman, J. G.
1905. Azotobacter studies. N. J. Agr. Expt. Sta. Ann. Rpt. (1905) 18:254–280.
(14) Löhnis, F., and Green, H. H.
1914. Über die entstehung und die zersetzung von humus, sowie über dessen einwirkung auf die stickstoff-assimilation. Centbl. Bakt. [etc.] (II) 40:52–60.
(15) Murray, T. J.
1917. Part I. The effect of different plant tissues on the fixation of atmospheric nitrogen. Va. Agr. Expt. Sta. Tech. Bul. 15: 93–102.
(16) Reed, H. S., and Williams, B.
1915. The effect of some organic soil constituents upon nitrogen fixation by azotobacter. Va. Agr. Expt. Sta. Tech. Bul. 4, p. 81–95.
(17) Stoklasa, J.
1908. Beitrag zur kenntnis der chemischen vorgänge bei der assimilation des elementaren stickstoffs durch azotobacter und radiobacter. Centbl. Bakt. [etc.] (II) 21:484–509, 620–632, illus.
(18) Van Slyke, D. D.
1912. The quantitative determination of aliphatic amino groups. II. Jour. Biol. Chem. 12:275–284, illus.
(19) Winters, N. E.
1924. Soil conditions which promote nitrogen fixation. Jour. Amer. Soc. Agron. 16:701–716.
(20) Zoond, A.
1926. The relation of combined nitrogen to the physiological activity of azotobacter. Brit. Jour. Expt. Biol. 4:[105]–113, illus.

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THE EFFECT OF THE AMOUNT OF FEED CONSUMED BY CATTLE ON THE UTILIZATION OF ITS ENERGY CONTENT[1]

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INTRODUCTION

The problem of assessing the value of a feed as a source of energy in nutrition, either in terms of net energy, total digestible nutrients, or other conceptions possessing an absolute rather than a comparative significance, is complicated by the fact that the utilization of the feed energy, either in digestion or in metabolism or both, is conditioned to some extent by the amount of feed consumed. Considerable evidence exists in the literature proving, for rations consisting entirely or to a large extent of concentrates, that digestion is more complete at low levels of intake than at high levels. For this reason, it has frequently been noted in experiments planned so as to allow definite amounts of digestible nutrients to the experimental animals, these amounts to be estimated on the basis of average digestion coefficients, that the digestible nutrients actually consumed, as determined in later digestion trials, are considerably smaller in amount than expected. The reason is that many if not most digestion coefficients have been determined at low levels of feeding.

The amount of food consumed has also been shown to affect the proportion of the food energy lost as animal heat, the so-called "heat increment," or the specific dynamic effect. In order to alleviate to some extent the discomfort of experimental human subjects undergoing basal metabolism measurements, occasioned by abstention from food for 12 to 15 hours, Soderstrom, Barr, and DuBois (*20*)[2] and later Benedict and Benedict (*3*) have studied the effects on heat production of small breakfasts containing 200 to 250 calories. It was found by the former investigators that, after one hour from the time of eating, the effect of the small meal on heat production was inconsiderable; the latter investigators were not able to establish any consistent effect of their meal on two subjects. With one subject, a woman, the metabolism was certainly not raised by the ingestion of food in two tests, even at 15 minutes after eating; in a third test it was raised from 5 to 7 per cent for a period of 40 to 60 minutes. With the second subject, a man, in the only test made, the influence of eating amounted to somewhat more than 10 per cent in the first 15-minute period, but at the time of the next determination, 40 minutes after eating, basal heat production had again established itself.

In two publications appearing in 1928 (*9*) and 1930 (*10*), Forbes and his associates reported the results of experiments on four steers, designed to measure the effect on heat production of increasing a

[1] Received for publication Oct. 9, 1931; issued August, 1932.
[2] Reference is made by number (italic) to Literature Cited, p. 190.

constant ration of corn meal and alfalfa hay, consisting of equal parts of dry matter from each feed, from a half-maintenance to a twice-maintenance or, in one case, to a thrice-maintenance level. They confirmed the results of Soderstrom, Barr, and DuBois and of Benedict and Benedict indicating an inappreciable effect on heat production of the ingestion of small amounts of food. From a level of 1.5 times maintenance upward, the heat production curves were approximately linear, though slight depressions in the curves, of uncertain significance, occurred at different levels of feeding. The investigators themselves describe the curve of heat production in relation to the plane of nutrition as a reversed or S curve. However, the significance of the downward trends at the higher levels of nutrition may be questioned, both because they are in all cases slight and because they occur at different levels of feeding when noticeable at all. In steer No. 47 a slight bend in the curve may be noted at a level of 1.5 times maintenance, but in its mate, steer No. 36, this bend is hardly perceptible. For steer No. 57 a bend in the curve occurs at a level of twice maintenance, but for steer No. 60 it is deferred until a level 2.5 times the maintenance requirements is reached.

Wiegner and Ghoneim (*21*), using a rabbit for their experiments, have reported an investigation of the utilization of the metabolizable energy of a constant ration at five different levels of intake. Only the highest level of feeding permitted a positive energy balance. The net energy of each level of food was computed as the difference between the energy balance of the period and the energy balance of fasting. The percentage net availability of the metabolizable energy decreased progressively from the lowest to the highest level of feeding, and the relation between intake of metabolizable energy and intake of net energy was a linear one. All data cited are expressed in terms of calories per square meter of body surface, and no reason or defense is given for this unique method of expression.

The relation of heat production to the level of feeding at high levels is of considerable practical importance in animal nutrition, corresponding as these levels do to the most productive feeding. The Pennsylvania experiments throw considerable light upon this relationship, and the experiments to be described below are concerned with the same subject. They were started before the Pennsylvania work was published.

DESCRIPTION OF THE EXPERIMENT

The purpose of the experiment to be described in this report is much the same as that of the Pennsylvania experiments, namely, to determine the effect of the amount of feed consumed upon the utilization of its energy. Four grade Shorthorn steers, approximately 2 years of age, were started on experiment, but since only one of them could be induced to consume enough feed under laboratory conditions to warrant continued study, the other three were soon discarded.

It was planned to select an experimental ration solely on the basis of its palatability. The ration finally selected, in the amounts constituting a full feed as determined by the animal's capacity to consume it, contained 10 kg of corn, 3 kg of alfalfa hay, 250 g of linseed meal, and 116 g of molasses. On the dry-matter basis, this ration contained, as the average of analyses in the five experimental

periods, 73.1 per cent of ground corn, 24.1 per cent of alfalfa hay, 2.0 per cent of linseed meal, and 0.8 per cent of molasses.

The five experimental periods involve observations on the digestibility and energy utilization of this ration at levels of full feed, as defined above, four-fifths feed, three-fifths feed, two-fifths feed, and one-fifth feed. The fasting metabolism of the steer was determined in two additional periods.

The chronological order of the experimental periods, their lengths and pertinent dates, all in the winter and spring months of 1928, are given in Table 1.

TABLE 1.—*Schedule of experiments*

Ration fed (grams)				Time on feed		
Corn	Alfalfa hay	Linseed meal	Molasses	Initial date	Final date	Days
8,000	2,400	200	100	Jan. 19	Feb. 14	27
10,000	3,000	250	116	Feb. 17	Mar. 11	[a] 21
6,000	1,800	150	75	Mar. 20	Apr. 5	17
0	0	0	0			
4,000	1,200	100	50	Apr. 20	May 10	21
2,000	600	50	25	May 11	May 31	21
0	0	0	0			

Collection period			Days in respiration chamber			
Initial date	Final date	Days	First dates	Second dates	Third dates	Experiment No.
Jan. 30	Feb. 12	14	Feb. 1–2	Feb. 3–4	Feb. 13–14	7, 8, 9.
Mar. 7	Mar. 11	5	Feb. 28–29	Mar. 1–2	Mar. 9–10	10, 11, 12.
Mar. 27	Apr. 5	10	Mar. 30–31	Apr. 2–3	Apr. 5–6	13, 14, 15.
			Apr. 16–17	Apr. 17–18	Apr. 19–20	16, 17, 18. [b]
May 1	May 10	10	May 2–3	May 8–9	May 9–10	19, 20, 21.
May 20	May 29	10	May 21–22	May 22–23	May 23–24	22, 23, 24.
			June 4–5	June 5–6	June 6–7	25, 26, 27. [b]

[a] Off feed on Mar. 2 to 4, inclusive.

[b] Fourth, fifth, and sixth days of fast.

Each experimental period consisted of a preliminary feeding period at least 7 days in length, a period of 10 to 12 days' duration in which the feces and urine were collected for analysis, and 3 days, consecutive or intermittent, in the respiration chamber. The amount of feed consumed was kept constant throughout each experimental period. These statements do not apply entirely to the period on full feed, since it was found difficult to keep the steer continuously on this high intake. The collection period in this case was of necessity reduced to 5 days, and the last day in the respiration chamber was marred by the refusal of part of the ration.

The collection periods in these experiments are shorter than those at times employed in metabolism work with cattle. At the Institute of Animal Nutrition of the Pennsylvania State College, for example, collection periods of 17 to 21 days are commonly used, while Møllgaard (*17*) advocates even longer periods. A 7-day preliminary feeding period is also considered inadequate by this investigator, although in the recently published experiments of Forbes and his associates, the preliminary feeding periods were frequently as short as 4 or 5 days. Judging by the results obtained at this laboratory there appears to be

no necessity for long preliminary periods or long collection periods in evaluating the digestibility or the metabolizable energy of rations for cattle, although in the evaluation of nitrogen or mineral balances characteristic of a given nutritive régime the necessity for longer periods seems much greater.

The speed with which the digestive tract of the ruminant adjusts itself to a new feed mixture is indicated by some digestion experiments performed in this laboratory on two sheep and four steers, carried on according to the chromic oxide method of Hedin or a modified method in which ferric oxide was taken as the inert material. The two sheep were receiving daily in two feedings 2 pounds of chopped alfalfa hay. After a number of days on hay alone, each sheep was given in addition 1.585 g of chromic acid, dispersed in an agar-agar jell and divided equally between the two feedings. The feces were collected daily and analyzed for dry matter and chromium. Chromium appeared in the feces of the first collection and increased in concentration up to the fourth day; from this time on the ratio of chromium to fecal dry matter maintained a level, the dispersion of the ratios for individual days around the mean of 18 days being expressed by coefficients of variation of 7.13 and 7.84. At the end of 18 days of feeding, the chromic oxide dosage was discontinued, and on the third day thereafter chromium had practically disappeared from the feces, the concentration decreasing from 0.222 to 0.006 per cent in one case and from 0.174 to 0.0004 per cent in the other. The experiments with steers gave essentially the same results, i. e., a rapid attainment of a fairly constant ratio of fecal dry matter to inert metal, a maintenance of this ratio as long as the inert material was fed with a constancy of 6 to 10 per cent (average 7.5 per cent) expressed as the coefficient of variation, and a rapid disappearance of the inert metal from the feces following its removal from the ration.

TABLE 2.—*Comparison of digestion coefficients obtained in 14-day and in 3-day collection periods on alfalfa and on timothy hay*

Steer No.	Length of collection period days	Coefficients of digestibility				
		Dry matter	Crude protein	N-free extract	Crude fiber	Ether extract
ALFALFA HAY						
1	14	59	70	66	52	60
	3	59	69	66	53	52
2	14	54	65	62	47	−2
	3	56	67	65	45	40
3	14	59	67	67	54	54
	3	60	69	66	55	45
4	14	56	69	65	46	26
	3	51	68	58	42	0
Averages	14	57.0	67.8	65.0	49.8	34.5
	3	56.5	68.3	63.8	48.8	34.3
TIMOTHY HAY						
1	14	50	12	56	53	26
	3	50	14	56	53	33
2	14	58	40	64	61	50
	3	56	39	62	58	53
4	14	54	46	59	53	52
	3	53	47	50	52	54
Averages	14	54.0	32.7	59.7	55.7	42.7
	3	53.0	33.3	56.0	54.3	46.7

Evidence of the same significance was obtained by the use of the ordinary method of determining digestibility. Four steers were fed in trials on alfalfa hay and three in trials on timothy hay. In each trial there was a preliminary period of 14 days followed by a collection period of 14 days. The feces of the first 3 days were aliquoted for incorporation into two samples, one for 3 days and the other for the full 14 days, and coefficients for both periods were determined. The results are assembled in Table 2.

For two steers on each hay, complete data were obtained for the computation of the metabolizable energy in the 3-day and 14-day collection periods, with the exception of methane excretion, which was estimated by the use of Armsby's factor of 4.5 g of methane per 100 g of digestible carbohydrates consumed. For the alfalfa hay the values in calories per kilogram of dry matter were 2,103 and 2,066 for the 14-day period, and 2,075 and 2,130, for the 3-day period, representing differences of −1.35 and +3.00 per cent. For the timothy hay the values were 1,858 and 1,824 for the 14-day periods and 1,849 and 1,776 for the 3-day periods, the differences being −0.49 and −2.70 per cent.

These data are presented, not with the idea of advocating such a short collection period as 3 days in digestion trials with steers, but in support of the general proposition that the need for long periods of 20 days or more is probably greatly exaggerated. The 10-day and 14-day periods used in the experiments reported below were long enough, the writers believe, to give accurate determinations of the metabolizable energy content of the rations, especially in view of the fact that examination of the daily records of fecal excretion revealed no marked irregularities at the beginning and end of the periods. The 5-day collection period on the highest level of feeding, necessitated by the refusal of the steer to consume this heavy ration for a longer time, might well be questioned were it not for the remarkable regularity in fecal excretion that obtained throughout. The daily weights of fresh feces during this period were 12.21, 12.05, 12.81, 14.24, and 12.38 kg, averaging 12.74 kg.

The respiration chamber used in these experiments (fig. 1) is of the open-circuit type. The principle of its construction and operation is the same as that of the chamber at the New Hampshire Agricultural Experiment Station described in bulletin 240 of that station (*19*), and of several respiration chambers used in the study of human metabolism at the nutrition laboratory of the Carnegie Institution of Washington, located at Boston. The outgoing air is aliquoted according to the Benedict principle, the size of aliquot being determined by the size of disk through which the main portion of the outgoing air passes from the apparatus. The size of aliquot obtained with each disk is carefully standardized by introducing pure carbon dioxide into the wind chest and determining the amount recovered from the sampling cans. The aliquoting table (fig. 2) accommodates 2 trains of bottles below for the absorption of water, 4 trains of bottles for the absorption of carbon dioxide, and 2 dry gas meters to measure the volume of the samples. This arrangement permits the taking of two simultaneous samples of chamber air. In this series of experiments the average difference between the simultaneous 24-hour samples of CO_2 was 1.16 per cent, 65 per cent of the

differences being less than 1 per cent and 85 per cent being less than 2 per cent.

The Illinois apparatus differs from the New Hampshire apparatus mainly in the construction of the chamber. In the Illinois apparatus

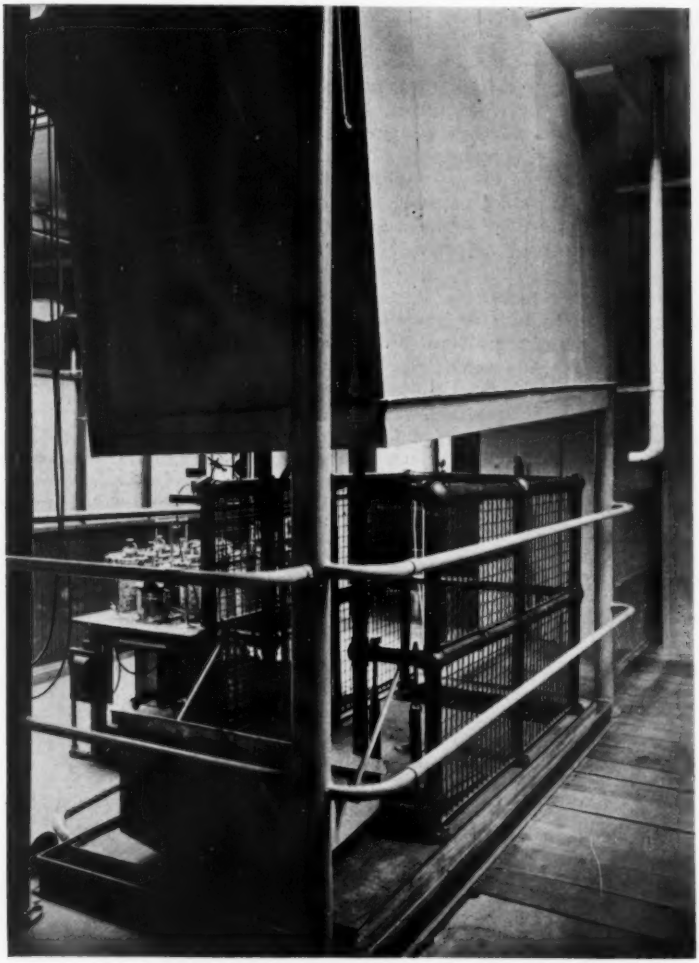

FIGURE 1.—General view of respiration chamber with cover up

the chamber consists of a base supporting a heavy iron stall framework, with a stanchion and a manger, containing a water trough which may be filled from the outside. A water seal completely surrounds the base of the chamber, and receives the heavy metal box constituting the walls and ceiling of the chamber and containing at the front an opening guarded by a cover sunk in a water seal

through which feed may be dumped into the manger. To admit the steer the metal box is raised to the ceiling of the room 14 feet from the floor by a system of ropes and pulleys, the process being facilitated by counterweights concealed in the four metal posts located at the four corners of the chamber. The reverse process secures the steer in an air-tight chamber from which connections are made with the incoming air pipe leading through the roof of the room to the outside, and with the outgoing air pipe leading to the wind chest on the aliquoting table. The ventilating fan is located at the exit opening, while another smaller fan at the upper front end of the metal box circulates the air in the chamber and prevents the formation of a

FIGURE 2.—Aliquoting table used with respiration chamber

dead air pocket. The volume of the chamber when containing a 1,000-pound steer is 6,581 liters.

The chamber is lighted electrically and contains a thermometer, a hygrometer, and a work adder attached to the movable platform on which the front feet of the steer rest. All of these instruments may be read from the glass window in the side. A pneumograph attached to the movable platform and extending through the base to the necessary recording arrangements supported on a shelf over the aliquoting table, permits the taking of a graphic record of the up and down excursions of this platform, measuring, albeit roughly, the activity of the steer. The chamber also contains an automatic device that rings a bell whenever the steer changes his position from standing to lying or the reverse.

The base of the chamber contains openings for the collection of feces and urine in a basement beneath, the former in a metal container suspended from the base and connected with it through a water seal, the latter in a brass cylinder into which the urine hose extends and from which the urine flows, through a water seal, into a glass carboy.

The apparatus itself permits of the continuous determination of the carbon dioxide produced by the steer. This was effected in six 4-hour subperiods. In order to determine the respiratory quotient, a continuous sample of air was taken at a constant rate from the outgoing air pipe and stored for each 4-hour subperiod in a gasometer with a capacity of approximately 100 l. Each sample collected in this fashion was then analyzed for carbon dioxide and oxygen in a Carpenter-Haldane analyzer (*8*). In addition, samples of air were taken during a 10-minute interval at the start of the experiment and at the end of each subperiod in order to determine the carbon dioxide content of the chamber to be used in computing the CO_2 production for the subperiods. The gas analyzers were checked each day by analyses of outdoor air and were not used until the results obtained fell within the limits 0.030 ± 0.003 for CO_2 and 20.940 ± 0.005 for O_2.

TABLE 3.—*The ratio of methane produced to digestible carbohydrates consumed by steers, at various levels of feeding, from the published experimental data of the Institute of Animal Nutrition, Pennsylvania State College*

Steer No.	Period No.	Ration	Plane of nutrition	Methane produced	Methane per 100 g. digestible carbohydrates
				Grams	
60	1	Alfalfa+corn	Maintenance	79.5	4.51
57	2	Alfalfa+corn	Maintenance	95.3	4.96
47	7	Alfalfa+corn	Maintenance	108.6	4.61
36	8	Alfalfa+corn	Maintenance	111.0	4.76
57	12	Alfalfa alone	Maintenance	119.4	5.14
60	13	Alfalfa alone	Maintenance	109.8	4.95
47	9	Alfalfa alone	Maintenance	125.7	5.03
36	10	Alfalfa alone	Maintenance	124.1	4.96
Average					4.86
60	3	Alfalfa+corn	1.5 maintenance	111.4	4.32
57	4	Alfalfa+corn	1.5 maintenance	133.5	4.60
47	3	Alfalfa+corn	1.5 maintenance	140.9	4.13
36	4	Alfalfa+corn	1.5 maintenance	138.0	4.24
Average					4.32
60	5	Alfalfa+corn	2.0 maintenance	156.3	4.54
57	6	Alfalfa+corn	2.0 maintenance	175.0	4.57
47	1	Alfalfa+corn	2.0 maintenance	183.2	4.19
36	2	Alfalfa+corn	2.0 maintenance	162.5	3.92
Average					4.30
60	7	Alfalfa+corn	2.5 maintenance	188.7	4.19
57	8	Alfalfa+corn	2.5 maintenance	219.6	4.56
Average					4.37
60	9	Alfalfa+corn	3.0 maintenance	230.4	4.22
47	5	do	0.5 maintenance	62.0	5.41
36	6	do	0.5 maintenance	62.3	5.42
57	10	do	0.5 maintenance	55.8	5.30
60	11	do	0.5 maintenance	55.3	5.36
Average					5.37

In each respiration experiment the steer was put into the chamber at 8 a. m., at which time one-half of the day's feed was put before him in the manger. The ventilation was not started until 8.15, in order that the carbon dioxide content of the chamber might reach, approximately, the level maintained throughout the day. When the ventilation was started a sample of air was taken for analysis of CO_2. The other half of the day's feed was given at 4 p. m. The steer was given measured amounts of water after each feeding.

During the entire experiment two men were always in attendance, and every half hour they recorded the temperature and humidity in the chamber, the position of the steer, the reading of the work adder, and the reading of the gas meters.

The methane content of the outgoing air was not determined. In the computation of the metabolizable energy content of the rations, the true carbon dioxide and oxygen content of the chamber air, and the heat production of the steer, the production of methane was estimated from the intake of digestible carbohydrates. Armsby has recommended a factor of 4.5 g of CH_4 per 100 g of digestible carbohydrates. However, in view of the fact that the level of feeding differed widely in the different experimental periods and that the methane production per unit of digestible carbohydrates may be presumed to vary with the level of feeding, especially at low levels, the relation of this factor to the level of feeding was investigated from the recently published experimental data of Forbes and his associates (*9*, *10*). In Table 3, computations of the ratio of methane produced to digestible carbohydrates consumed, made from the Pennsylvania data, have been summarized.

It appears from the computations that the ratio is not subject to any appreciable change from levels of feeding ranging from 1.5 to 3.0 times the maintenance level. At the lower levels of feeding, however, the ratio increased considerably. Therefore, for the periods in the present experiment involving full feed, four-fifths feed, and three-fifths feed, the writers have continued to use the factor of 4.5 g of methane per 100 g of digestible carbohydrates in preference to a lower factor of 4.3, favored by the data in Table 3, since it is based upon somewhat more data and since the difference between the two factors is inappreciable in its effect on the calculations in this experiment. Since the two-fifths full feed level proved to be close to a maintenance ration, a factor of 4.85 g of methane per 100 g of digestible carbohydrates was used in this period, and a factor of 5.5 g was used in the one-fifth full feed period.

RESULTS OF THE EXPERIMENT

THE BODY WEIGHTS OF THE STEER

The body weights of the steer at the beginning and end of each collection period and at the time of each confinement in the respiration chamber are given in Table 4. Each weight given is the average for three consecutive days. When respiration experiments were undertaken on three consecutive days, only two average weights are given, one for the morning on which the steer entered the chamber and the two preceding mornings, and the other for the morning on which the steer left the chamber and the two following mornings.

TABLE 4.—*Body weights of the steer in kilograms at beginning and end of each collection period and at time of each confinement in the respiration chamber*

Plane of nutrition	Collection period		Respiration experiments		
	Initial weight [a]	Final weight [a]	First weight [a]	Second weight [a]	Third weight [a]
Four-fifths full feed	582.0	594.2	591.5	586.1	597.4
Full feed	611.5	622.8	621.4	625.5	622.3
Three-fifths full feed	624.2	627.3.	624.6	632.8	627.3
Fasting			632.3		623.7
Two-fifths full feed	610.1	615.1	614.6	607.8	615.1
One-fifth full feed	604.6	588.8	604.6		599.7
Fasting			603.3		602.4

[a] Each weight given represents an average for 3 consecutive days.

THE DIGESTIBILITY OF THE RATION AT DIFFERENT LEVELS OF FEEDING

The complete data relating to the calculation of the digestion coefficients of dry substance, crude protein, nitrogen-free extract, crude fiber, and ether extract are assembled in Table 5. To facilitate comparison, the coefficients have been summarized in Table 6. Table 7 gives the average composition of the feeds used.

TABLE 5.—*The calculation of digestion coefficients of various ration constituents at different levels of feeding*

FULL FEED

Item	Direct weight	Dry substance	Crude protein	N-free extract	Crude fiber	Ether extract
Alfalfa ..... grams	3,000	2,761	429	1,215	786	105
Corn ..... do	10,000	8,159	787	6,663	223	363
Linseed meal ..... do	250	228	92	91	20	11
Molasses ..... do	116	81	5	66	.....	.....
Total ..... do	.....	11,229	1,313	8,035	1,029	479
Feces ..... do	12,739	2,954	463	1,572	512	190
Nutrients digested ..... do	.....	8,275	850	6,463	517	289
Digestibility ..... per cent	.....	73.7	64.7	80.4	50.2	60.3

FOUR-FIFTHS FULL FEED

Alfalfa ..... grams	2,400	2,209	335	966	624	58
Corn ..... do	8,000	6,514	655	5,250	243	266
Linseed meal ..... do	200	182	73	72	17	10
Molasses ..... do	100	70	5	57	.....	.....
Total ..... do	.....	8,975	1,068	6,345	884	334
Feces ..... do	10,879	2,300	409	1,102	506	101
Nutrients digested ..... do	.....	6,675	659	5,243	378	233
Digestibility ..... per cent	.....	74.4	61.7	82.6	42.8	69.8

THREE-FIFTHS FULL FEED

Alfalfa ..... grams	1,800	1,627	244	694	507	50
Corn ..... do	6,000	4,990	465	4,100	151	205
Linseed meal ..... do	150	136	55	55	12	8
Molasses ..... do	75	53	3	43	.....	.....
Total ..... do	.....	6,806	767	4,892	670	263
Feces ..... do	7,440	1,552	315	709	306	72
Nutrients digested ..... do	.....	5,254	452	4,183	364	191
Digestibility ..... per cent	.....	77.2	58.9	85.5	54.3	72.6

TWO-FIFTHS FULL FEED

Alfalfa ..... grams	1,200	1,115	172	482	318	45
Corn ..... do	4,000	3,402	320	2,753	120	154
Linseed meal ..... do	100	92	36	37	8	5
Molasses ..... do	50	35	2	29	.....	.....
Total ..... do	.....	4,644	530	3,301	446	204
Feces ..... do	4,167	888	196	336	212	40
Nutrients digested ..... do	.....	3,756	334	2,965	234	164
Digestibility ..... per cent	.....	80.9	63.0	89.8	52.5	80.4

TABLE 5.—*The calculation of digestion coefficients of various ration constituents at different levels of feeding*—Continued

ONE-FIFTH FULL FEED

Item	Direct weight	Dry substance	Crude protein	N-free extract	Crude fiber	Ether extract
Alfalfa.....grams..	600	549	96	229	154	13
Corn.....do....	2,000	1,710	156	1,386	65	76
Linseed meal.....do....	50	46	18	19	4	2
Molasses.....do....	25	18	1	14	----	----
Total.....do....	----	2,323	271	1,648	223	91
Feces.....do....	1,738	373	87	121	86	14
Nutrients digested.....do....	----	1,950	184	1,527	137	77
Digestibility.....per cent..	----	83.9	67.9	92.7	61.4	84.6

TABLE 6.—*Summary of digestion coefficients (per cent) of various ration constituents at different levels of feeding*

Plane of nutrition	Dry substance	Crude protein	N-free extract	Crude fiber	Ether extract
Full feed	73.7	64.7	80.4	50.2	60.3
Four-fifths full feed	74.4	61.7	82.6	42.8	69.8
Three-fifths full feed	77.2	58.9	85.5	54.3	72.6
Two-fifths full feed	80.9	63.0	89.8	52.5	80.4
One-fifth full feed	83.9	67.9	92.7	61.4	84.6

TABLE 7.—*Average percentage chemical composition of feeds used*

Feed	Dry substance	Crude protein	N-free extract	Crude fiber	Ether extract	Ash	Gross energy
							Calories per gram
Alfalfa hay	91.79	14.42	39.53	26.52	2.94	8.37	3.97
Corn	83.35	7.92	67.75	2.80	3.60	1.28	3.68
Linseed meal	91.23	36.49	36.81	7.93	4.81	5.20	4.31
Molasses	70.26	4.51	57.13	----	----	8.62	2.64

The lowest level of feeding was associated with the most complete digestibility of all nutrients. However, there was a progressive decrease in digestibility from the lowest to the highest ration only in the case of nitrogen-free extract, ether extract, and dry substance.

THE METABOLIZABLE ENERGY AT DIFFERENT LEVELS OF FEEDING

The computation of the total metabolizable energy consumed at the different levels of feeding is given in Table 8. The usual correction of the energy loss in the urine to a condition of nitrogen equilibrium has been made on the assumption that each gram of nitrogen retained by (or lost from) the body would on excretion in the urine (or retention in the body) carry with it 7.45 calories of nonmetabolizable energy. The metabolizable energy values thus obtained more nearly represent the actual content of metabolizable energy in the rations consumed. The nitrogen balances upon which this correction is based are given in Table 9.

TABLE 8.—*Calculation of the metabolizable energy, in Calories, of the ration at different levels of feeding*

Plane of nutrition	Gross energy of feeds consumed					Energy of feces	Energy of urine			Energy of methane	Total metabolizable energy
	Alfalfa hay	Corn	Linseed meal	Molasses	Total		Observed	Correction to nitrogen equilibrium [a]	Corrected		
Full feed	12,036	35,880	1,072	307	49,295	14,038	861	+326	1,187	4,189	29,881
Four-fifths full feed	9,708	28,896	849	264	39,717	11,020	729	+273	1,002	3,375	24,320
Three-fifths full feed	6,934	21,876	650	198	29,658	7,343	548	+56	604	2,729	18,982
Two-fifths full feed	4,808	15,032	436	132	20,408	4,209	503	+64	567	2,069	13,563
One-fifth full feed	2,368	7,598	216	66	10,248	1,564	319	−42	277	1,220	7,187

[a] See nitrogen balances given in Table 9.

TABLE 9.—*The daily nitrogen balances (grams) of the steer at different levels of feeding*

Plane of nutrition	Nitrogen in feed	Nitrogen in feces	Nitrogen in urine	Nitrogen excreted	Nitrogen balance
Full feed	210.21	74.19	92.31	166.50	+43.71
Four-fifths full feed	170.78	65.45	68.72	134.17	+36.61
Three-fifths full feed	122.75	50.43	64.59	115.02	+7.73
Two-fifths full feed	84.86	31.41	44.84	76.25	+8.61
One-fifth full feed	43.41	13.98	35.02	49.00	−5.59

A further study of the losses of nonmetabolizable energy and of the metabolizable energy remaining is permitted from the data summarized in Table 10. In particular, it is important to note that the metabolizable energy per kilogram of dry matter consumed, as well as the percentage metabolizability of the gross energy, increases progressively from the highest to the lowest level of feeding, the former from 2,661 Calories to 3,094 Calories, and the latter from 60.61 per cent to 70.14 per cent. However, when the metabolizable energy is computed to a kilogram of digestible nutrients, all effect of the level of feeding disappears.

TABLE 10.—*Further computations with reference to the metabolizable energy of rations*

Plane of nutrition	Dry matter eaten daily	Digestible nutrients eaten daily	Energy per kilogram of dry matter					Metabolizable energy per kilogram of total digestible nutrients	Losses of energy			Metabolizable energy
			Total	Losses			Metabolizable		In feces	In urine	In methane	
				In feces	In urine	In methane						
	Kilograms	*Kilograms*	*Calories*	*Calories*	*Calories*	*Calories*	*Calories*	*Calories*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Full feed	11.229	8.480	4,390	1,250	106	373	2,661	3,524	28.47	2.41	8.50	60.61
Four-fifths full feed	8.975	6.002	4,425	1,228	112	376	2,709	3,575	27.75	2.53	8.50	61.22
Three-fifths full feed	6.806	5.426	4,358	1,074	88	401	2,795	3,498	24.64	2.02	9.20	64.13
Two-fifths full feed	4.644	3.899	4,394	906	122	446	2,920	3,478	20.62	2.78	10.15	66.45
One-fifth full feed	2.323	2.022	4,411	673	119	525	3,094	3,554	15.26	2.70	11.90	70.14

In view of the fact that the methane production was estimated, not directly determined, in this experiment, it is worthy of note that the energy lost as methane amounted to only 8.5 to 11.9 per cent of the gross energy consumed. Hence, even a considerable percentage error in the estimation of the methane production may exert only an inappreciable error on the computation of the metabolizable energy values.

THE ENERGY METABOLISM OF THE STEER

The results of the respiration experiments have been assembled in Table 11. The average respiratory quotients have been obtained from the average gas analyses of air samples collected in the six subperiods, after correction for the estimated methane content of the chamber air. The volume of oxygen consumed was obtained from the volume of carbon dioxide produced and the respiratory quotient.

TABLE 11.—*The results of the respiration experiments*

Date of experiment	Live weight of steer	Dry matter consumed	Chamber temperature		Chamber humidity		Hours standing out of 24	Average respiratory quotient	Carbon dioxide produced		Oxygen consumed
			Average	Range	Average	Range			Grams	Liters	
	Kilograms	*Grams*	*° C.*	*° C.*	*Per cent*	*Per cent*					*Liters*
Feb. 28–29	621.4	11,229	22.9	21.5–25.0			14.33	1.16	8,692	4,425	3,815
Mar. 1–2	625.5	11,229	21.6	21.0–22.2	93	81–98	12.13	1.12	8,194	4,172	3,725
Mar. 9–10	622.3	[a]11,229	21.1	18.9–22.2	95	90–98	13.72	1.11	7,863	4,003	3,606
Feb. 1–2	591.5	8,976	23.7	22.5–24.5			16.55	1.14	7,242	3,687	3,234
Feb. 3–4	586.1	8,976	24.5	23.0–24.8			12.82	1.11	6,786	3,455	3,113
Feb. 13–14	597.4	8,976	22.8	21.8–23.5			15.47	1.12	7,051	3,590	3,205
Mar. 30–31	624.6	6,807	18.6	17.2–20.0	89	77–99	16.85	1.03	6,137	3,124	3,033
Apr. 2–3	632.8	6,807	23.1	21.6–25.0	93	87–97	8.27	1.08	5,789	2,947	2,729
Apr. 5–6	627.3	6,807	22.8	22.2–23.3	95	89–99	12.87	1.04	6,124	3,118	2,998
May 2–3	614.6	4,644	22.8	21.6–25.5	83	71–92	11.38	.95	4,745	2,416	2,543
May 8–9	607.8	4,644	22.8	21.1–25.6	76	65–87	11.35	.97	4,887	2,488	2,565
May 9–10	615.1	4,644	23.9	22.2–26.7	87	78–93	11.98	.98	4,968	2,529	2,581
May 21–22	604.6	2,323	25.6	21.8–27.2	86	83–90	13.60	.87	3,574	1,820	2,092
May 22–23		2,323	23.3	21.6–25.0	85	81–88	11.10	.88	3,436	1,749	1,988
May 23–24	599.7	2,323	22.8	21.1–25.0	80	79–84	11.35	.89	3,491	1,777	1,997
Apr. 16–17	632.3	0	20.0	17.2–21.6	72	66–78	12.43	.70	2,887	1,470	2,100
Apr. 17–18		0	20.6	20.0–21.1	93	90–96	9.03	.71	2,801	1,426	2,008
Apr. 18–19	623.7	0	21.6	20.6–22.8	91	89–94	9.35	.71	2,761	1,406	1,980
June 4–5	603.3	0	20.6	20.0–21.1	97	94–100	12.68	.69	2,765	1,408	2,011
June 5–6		0	21.1	20.6–22.8	95	91–97	12.62	.68	2,711	1,380	1,971
June 6–7	602.4	0	21.1	20.0–21.6	95	92–98	13.30	.68	2,717	1,383	1,976

[a] Considerable feed was refused on this day.

In Table 12 the respiratory quotients of the six subperiods in each experiment are given. The gas analyses at the lowest plane of nutrition were in error in a large portion of the cases, although at least one good respiratory quotient was available for each experimental subperiod of four hours. The average respiratory quotient for the day at this level was estimated as accurately as possible from the reliable analyses available, supplemented by information concerning the CO_2 content of the chamber air obtained from the continuous gravimetric determination of carbon dioxide on the aliquoting table and the total volume of air withdrawn from the chamber.

It is evident from the values given in Table 12 that (1) at the two highest levels of feeding the respiratory quotient was continuously greater than 1, (2) at the level of three-fifths full feed the quotient was above 1 for approximately eight hours after each feeding, (3) at the level of two-fifths full feed a quotient greater than unity did not obtain for more than four hours after feeding, and (4) at the lowest

level of feeding no quotients greater than unity were observed, although such quotients may have obtained for a short period after feeding. At all levels of feeding except the lowest, the respiratory quotient for the first four hours after feeding was higher than for any other period, and progressively decreased at the higher levels of feeding, less clearly at levels lower than these.

TABLE 12.—*The respiratory quotients obtained during the six subperiods in each experiment, together with the average quotients for the day*

Plane of nutrition	Experiment No.	Respiratory quotient during indicated hours after feeding						Total respiratory quotients for the day		
		1 to 4	4 to 8	1 to 4	4 to 8	8 to 12	12 to 16	Observed	Corrected for methane [a]	Corrected to complete oxidation [b]
Full feed	10	1.19	1.13	1.22	1.09	1.08	1.04	1.13	1.16	1.05
	11	1.13	1.04	1.13	1.10	1.08	1.02	1.08	1.12	1.01
	12	1.06	1.09	1.10	1.09	1.03	1.09	1.08	1.11	1.00
Average		1.13	1.09	1.15	1.09	1.06	1.05	1.10	1.13	1.02
Four-fifths full feed	7	1.16	1.09	1.14	1.10	1.05	1.10	1.11	1.14	1.03
	8	1.16	1.04	1.20	1.04	1.05	1.00	1.08	1.11	1.01
	9	1.13	1.08	1.21	1.06	1.05	.99	1.09	1.12	1.02
Average		1.15	1.07	1.18	1.07	1.05	1.03	1.09	1.12	1.02
Three-fifths full feed	13	.97	1.05	1.12	.99	.96	.97	1.01	1.03	.96
	14	1.12	1.01	1.17	1.00	1.04	.95	1.05	1.08	.99
	15	1.01	1.04	1.09	1.03	.98	.96	1.02	1.04	.96
Average		1.03	1.03	1.13	1.01	.99	.96	1.03	1.05	.97
Two-fifths full feed	19	.95	.99	.....	.90	.85	.96	.93	.95	.89
	20	.92	.98	1.10	.85	.88	.95	.95	.97	.91
	21	1.17	.92	1.03	.89	.90	.89	.96	.98	.92
Average		1.01	.96	1.06	.88	.88	.93	.95	.97	.91
One-fifth full feed	22	.....	.....	.90	.90	.....	.....	.86	.87	.84
	23	.87	.87	.91	.91	.83	.....	.87	.88	.84
	24	.....	.....	.93	.95	.....	.81	.87	.89	.85
Average		.87	.87	.91	.92	.83	.81	.87	.88	.84

[a] Gas analyses corrected for presence of methane.
[b] Assuming that the methane had been oxidized to CO_2 and H_2O.

The average respiratory quotients for the 24 hours were, in the order of decreasing levels of feeding, 1.10, 1.09, 1.03, 0.95, and 0.87. However, these quotients are to some extent in error, because of the methane production. The oxygen deficit in the chamber air can not be estimated accurately in the presence of appreciable quantities of methane without making due allowance for the latter; the CO_2 excess in the chamber air is not appreciably affected by the presence of methane in the concentration produced. From the estimated day's production of methane, based as explained above on the intake of digestible carbohydrates, the following volumes of methane produced for each liter of CO_2 were estimated, in the order of decreasing levels of feed intake, i. e., 0.0932, 0.0881, 0.0831, 0.0779, and 0.0639 liters. The average percentages of CO_2 in the expired air for 24 hours were, in the same order, 0.614, 0.534, 0.455, 0.387, and 0.305. From these two types of data the average percentages of methane in the chamber were estimated to be as follows, in the order of decreasing levels of feeding: 0.056, 0.046, 0.038, 0.030, and 0.019. The methane correction of the observed gas analysis values diminished the uncorrected

oxygen deficit in the expired air and increased the average respiratory quotients from the average values given above to the following average values, given in the usual order: 1.13, 1.12, 1.05, 0.97, and 0.88.

However, these respiratory quotients are not usable in the computation of heat production, since they do not correspond to complete oxidation of the food nutrients. To obtain usable respiratory quotients, the observed volumes of CO_2 produced and O_2 consumed must be increased by the volumes of these gases involved in the complete oxidation of the amounts of CH_4 estimated to have been produced. This has been done in computing the respiratory quotients given in the last column of Table 12. The heat production per 24 hours was estimated from the volume of oxygen consumed (Table 11) plus the volume of oxygen required to oxidize the methane formed. If the average respiratory quotient for the day, computed with reference to complete oxidation, was above unity, the heat equivalent of the oxygen was taken as 5.047 Calories per liter, the value for a respiratory quotient of unity. To allow for the heat evolved in the conversion of carbohydrates to fats, as indicated by a respiratory quotient above unity, the excess of CO_2 produced over the O_2 consumed was considered to represent an extra heat output of 0.803 Calorie per liter,[3] a factor that Lusk has computed from the equation of Bleibtreu for the conversion of glucose to fat. The heat production for the day was considered to be given, following the method of Anderson (*1*), by the sum of these two quantities of heat minus the heat of combustion of the methane produced. If the average respiratory quotient for the day was less than unity, the heat equivalent of the oxygen consumed plus that required to oxidize the methane was determined from the well-known tables of Lusk (*14*), and from the heat thus computed was deducted the heat equivalent of the methane.

It is interesting to consider the effect of these corrections for the production of methane and for its presence in the chamber air upon the computation of heat production. The average percentage errors incurred by a neglect of these corrections would be, for the different levels of feeding, +5.35, +4.87, +5.08, +5.63, and +2.04. The two corrections above described are of about equal importance in their effect upon the final computations.

In the method of computation just described the protein metabolism is entirely neglected, the total respiratory quotients being given the significance of nonprotein respiratory quotients in the determination of the caloric equivalent of each liter of oxygen consumed. There is a method in somewhat common use for making allowance for protein in these calculations by the use of certain correction factors, by which the CO_2 and heat produced in protein metabolism and the oxygen consumed are estimated from the nitrogen excreted in the urine. By using these factors, a nonprotein respiratory quotient may be computed in each respiration experiment.

[3] In the fourth edition of Lusk's book The Science of Nutrition (*14, p. 396*) a recalculation of this factor has been made, leading to a value of 1.09 calories per liter of CO_2 produced in the transformation of carbohydrate to fat. The writers' attention was called to this revision after all the calculations given in this paper were completed. When it was ascertained that the new factor would occasion an entirely insignificant change in the values for total heat production, less than 100 calories and generally considerably less, the task of making this revision was not undertaken. This method of evaluating the heat produced in the conversion of sugar to fat is based upon a hypothetical equation of Bleibtreu, and its validity is not universally admitted among investigators in animal and human calorimetry. It is an attempt to evaluate the *maximum* production of fat from sugar, and a number of other equations, involving different energy relations, have been proposed. It seems impossible to select the most probable equation for this transformation on the basis of any accurate information available.

The derivation of these factors is described in considerable detail by Lusk (*14*), and his factors are used rather widely in this country. It is a significant fact that they are based upon the composition of meat protein and upon the extent of its metabolizability in the dog. They are applied, however, not only to fasting animals, in which the protein katabolism relates mainly to muscle protein oxidation, but to animals on rations containing any variety of protein. In these cases the protein katabolism relates to dietary protein, and the relations of respiratory CO_2 and O_2 and of heat to the urinary nitrogen must be different from those calculated for meat protein. To apply meat-protein factors to such conditions is to assume that all proteins will give essentially the same factors, although it is known that the nitrogen content of vegetable proteins may vary considerably from that of meat protein. The relative indigestibility of vegetable proteins would complicate further the computation of factors such as those devised by Lusk for meat protein.

Magnus-Levy (*15*) has expanded upon this difficulty in the way of an accurate assessment of the share of protein in the respiratory metabolism of animals and has compromised by giving factors for protein which are the averages of values relating to "muscle substance" and to casein. According to Lusk, 1 liter of oxygen used in the combustion of protein is equivalent to 4.463 Calories of heat; according to Magnus-Levy it is equivalent to 4.578 Calories. Benedict and Joslin (*5*) have computed a value of 4.60 Calories for this factor, while Møllgaard's factors (*17*) lead to a value of 4.639 Calories. According to Lusk, each gram of nitrogen excreted in the urine represents 26.51 Calories of protein metabolism; according to Magnus-Levy, 27.14 Calories; and according to Møllgaard, 28.12 Calories.

It is evidently impossible to assess accurately the extent of the participation of protein in the respiratory and energy metabolism of animals from the excretion of nitrogen in the urine, and fortunately the need for this computation is not great, since the error consequent upon its neglect will never, under practical conditions of feeding, be considerable. This fact depends upon the further fact that the heat value of a liter of oxygen used in the combustion of protein appears to differ but slightly from the heat value of a liter of oxygen used in the oxidation of a mixture of fat and sugar having the same respiratory quotient as protein, i. e., 4.803 to 4.810, depending upon the precise respiratory quotient assigned to protein. The maximum error would obviously be made when the total observed respiratory quotient is 0.802 and when protein is the only nutrient undergoing oxidation, a condition theoretically possible but rarely if ever realized in actual nutrition. If, now, protein metabolism be entirely neglected and the total respiratory quotient be taken as a nonprotein respiratory quotient, then the error in the estimation of the heat value of each liter of oxygen consumed would, according to the values accepted by Lusk, be $4.803-4.463=0.340$ Calories, or 7.62 per cent. If Benedict's value for protein is taken, the maximum error would be $4.803-4.600=0.203$ Calories, or 4.41 per cent, while, if Møllgaard's value is used, an error of $4.803-4.639=0.164$ Calories, or 3.54 per cent would result.

This is evidently an extreme condition, since the protein metabolism for the day rarely exceeds 20 to 25 per cent of the total, and, according to Magnus-Levy, is generally no greater than 15 per cent

of the total. Also as the total respiratory quotient deviates from 0.80 the maximum possible protein metabolism rapidly decreases, as well as the maximum error realized by its neglect. The calculations in Table 13 illustrate this fact. Thus, for a total respiratory quotient of 0.95, it would be possible, in the event of no oxidation of fats, for 23 per cent of the heat produced to result from the oxidation of protein. However, if this unusual condition actually existed and if the computation of the heat production were made on the assumption that the total respiratory quotient was a nonprotein respiratory quotient, the result would be in error by only 1.7 per cent.

TABLE 13.—*The maximum error made in neglecting protein metabolism in the computation of the heat production from the respiratory exchange*

Total respiratory quotient	Maximum protein metabolism possible	Heat value of a liter of oxygen for maximum protein metabolism	Heat value of a liter of oxygen, neglecting protein metabolism	Maximum error in neglecting protein metabolism
	Per cent	*Calories*	*Calories*	*Per cent*
0.75	45	4.584	4.739	3.3
0.80	100	4.463	4.801	7.6
0.85	73	4.607	4.863	5.6
0.90	47	4.754	4.924	3.6
0.95	23	4.901	4.985	1.7

It would be extremely unlikely under any conditions that the maximum percentage of protein metabolism would ever be realized. If no more than 20 per cent of the total heat produced is derived from the oxidation of protein, a fairly high level, the maximum error committed by neglecting protein metabolism entirely would be about 1.5 per cent. In the present experiment the protein calories constitute less than 13 per cent of the total calories, as ordinarily computed from the nitrogen in the urine (Table 9), and the differences between the total heat production computed with and without a correction for protein metabolism, by using Lusk's factors, range from 1.25 per cent at the level of full feed to 0.69 per cent at the level of one-fifth full feed. It is true that it would be advisable to avoid errors even of this magnitude if a good method of doing so were at hand. It is probable that the uncorrected values are slightly too high, but there is no assurance that if Lusk's factors were used in correcting the heat production for protein metabolism the estimates obtained would not be slightly too low.

In view of the uncertainty in all methods proposed for the measurement of the participation of protein in the respiratory and energy metabolism of animals, and in view of the inconsiderable error that results apparently from the entire neglect of this point, the writers prefer to make their indirect calorimetric computations on the basis of total respiratory quotients rather than nonprotein respiratory quotients. This is the practice followed in much of the recent work in human metabolism and in most of the work reported from the nutrition laboratory of the Carnegie Institution of Washington, and defended on the basis of the small errors incurred (*4*).

The respiratory quotients obtained during the two fasting experiments, representing in each case observations obtained during the

fourth, fifth, and sixth days of fast, with no preliminary preparation of the steer, are given in Table 14. In the 4-hour samples of chamber air, respiratory quotients below that of fat were frequently obtained, and even the average gas analyses for the entire day indicated quotients slightly below 0.7 in half the cases. In these cases the heat production was computed on the assumption of 100 per cent fat metabolism and a heat equivalent of the consumed oxygen of 4.686 Calories per liter.

TABLE 14.—*Respiratory quotients obtained during two fasting experiments*

Experiment No.	Respiratory quotient in indicated 4-hour periods						Respiratory quotient for 24-hour period
	8 a. m. to 12 m.	12 m. to 4 p. m.	4 p. m. to 8 p. m.	8 p. m. to 12 p. m.	12 p. m. to 4 a. m.	4 a. m. to 8 a. m.	
16	0.71	0.74	0.66			0.63	0.70
17	.66	.73	.70	0.73	0.72	.75	.71
18	.72			.68	.67	.66	.71
25	.68	.68	.70	.69	.71	.70	.69
26	.68	.70	.67	.69	.68	.67	.68
27	.70	.68	.69	.69	.65	.69	.68

Before studying the final computations of the experiment, assembled in Table 17, it is advisable to consider the results of the fasting experiments to be found in Table 15. These computations of heat production did not involve any corrections for methane, since the methane production of the steer after the third day of fasting is inconsiderable (*9*, *10*).

TABLE 15.—*Heat production of the steer during the fourth, fifth, and sixth days of fast*

Body weight of steer	Body surface of steer	Date	Day of fast	Heat production			
				Observed	Per standard day		
					Total	Per square meter	Per 500 kilograms
Kilograms	*Square meters*			*Calories*	*Calories*	*Calories*	*Calories*
628.0	5.33	April 16–17	4	9,842	9,855	1,850	8,674
		April 17–18	5	9,366	9,590	1,800	8,441
		April 18–19	6	9,285	9,489	1,780	8,352
602.8	4.96	June 4–5	4	9,532	9,494	1,914	8,550
		June 5–6	5	9,514	9,480	1,911	8,537
		June 6–7	6	9,476	9,399	1,895	8,464

In correcting the observed heat production to a standard day of 12 hours each in the standing and lying positions, following the practice initiated by Armsby and his associates, a factor of 62 Calories per hour per 502 kg of body weight was taken as the extra heat production of standing over lying. This factor is the average of two values obtained during the preceding year on the same steer at a body weight of 500 kg and on another Shorthorn steer at a body weight of 447 kg, on the ninth and tenth days of fast, respectively. During these days

the hourly production of carbon dioxide was obtained for considerable periods of time in the standing and in the lying position. For the first steer the hourly CO_2 production while lying (4 periods of observation totaling 5.17 hours) was 81.07 g, and that while standing (4 periods totaling 11.79 hours) was 98.24 g, an increase of 21 per cent. In this experiment the increment in heat production due to standing amounted to 60 Calories per hour per 500 kg body weight. For the other steer the hourly production of CO_2 while lying (6 periods totaling 3.67 hours) was 81.75 g, and while standing (3 periods totaling 7.45 hours) 98.16 g, an increase of 20 per cent, or, in terms of heat production, 64 Calories per hour per 500 kg.

The surface area of the steer was determined at each of the fasts by the use of the Brody surface integrator (7). In the first fasting experiment the area [4] was 5.33 m^2, and in the second 4.96 m^2. These values are somewhat larger than those computed from Brody's formula for beef cattle $A = 0.13\ W^{.56}$ i. e., 4.80 and 4.69 m^2, respectively.

The fasting heat production (corrected to the standard day) per square meter of body surface averaged 1,810 Calories for the first fasting period and 1,907 Calories for the second fasting period, or, using the surface areas as determined by the Brody formula, 2,010 and 2,017 Calories, respectively.

In the respiration experiments of the preceding year, already referred to, short observations of 1.5 to 2.0 hours were made on the carbon dioxide production of four grade Shorthorn steers, including the one used in the present experiments, on the fourth or the fifth day of absolute fast. The heat production was calculated on the assumption of a fasting respiratory quotient of 0.72. The results of these experiments are given in Table 16, and relate to the standing position.

TABLE 16.—*The heat production of fasting steers on fourth and fifth days of the fast*

Steer No.	Body weight	Surface area [a]	Day of fast	CO_2 per hour	Heat production per 24 hours		
					Observed	Corrected to standard day [b]	
						Total	Per square meter of body surface
	Kilograms	*Square meters*		*Grams*	*Calories*	*Calories*	*Calories*
1	501.7	4.23	Fourth	106.7	8,507	7,761	1,835
2	447.2	3.96	do	100.6	8,021	7,356	1,858
3	433.6	3.90	Fifth	100.9	8,045	7,508	1,925
4	402.8	3.74	do	87.3	6,960	6,461	1,727

[a] Computed by the Brody formula, $A = 0.13\ W^{.56}$.
[b] Using the factor 62 Calories per hour per 500 kg of body weight for the excess heat produced in the standing position.

The values for the heat production per standard day per square meter of surface area are quite similar to those obtained in the later experiment on steer 1. They are, however, considerably greater than

[4] m^2 is the abbreviation for square meter recently adopted by the Style Manual for United States Government printing.

those given for fasting steers and dairy cows, by Forbes, Fries, Braman, and Kriss (*11*) and by Forbes, Kriss, and Braman (*12*), averaging approximately 1,400 Calories. In two recent publications from the Pennsylvania laboratory (*9, p. 276; 10, p. 51*) the fasting metabolism of the four steers used as subjects was 1,588, 1,628, 1,642, and 1,491 Calories per square meter of surface area. However, the former values were obtained by the use of Moulton's formula for the surface area of beef cattle (*18*), based upon the measured area of the removed hides of a number of animals, while the latter four values were based upon the area of the hides of the experimental animals themselves, removed and measured after slaughter. This method of determining surface area may be expected to give larger areas than those obtained by the Brody surface integrator from the live animals. The Moulton formula applied to the steer in the experiment under discussion gives areas of 6.22 and 6.07 m^2 for the two fasting periods, and heat productions per standard day per square meter of 1,550 and 1,558 Calories. It may be concluded, therefore, that the fasting heat productions of steers in the Illinois experiments are quite consistent with those obtained at the Pennsylvania laboratory when computed to the unit of surface area by the same formula. The average of 13 determinations of the fasting heat production of beef cattle, corrected to the standard day, from the Illinois and Pennsylvania experiments, is $1,897 \pm 19$ Calories per square meter of body surface, the latter computed according to the Brody formula. The standard deviation of these 13 determinations was 100 and their coefficient of variation 5.27.

The experiments of Benedict and Ritzman (*6*) on four fasting steers gave average values for the heat production per day (in the standing position) per square meter of surface of 1,474, 1,591, 1,590, and 1,570 Calories on the fourth and fifth (or the fourth or fifth) days of fast. These investigators have obtained such discordant values for the influence of standing on the heat production that they have not attempted a correction to the standard day. The surface areas of the steers were estimated in these experiments by the Hogan modification of the Moulton formula (*13*), namely, $A = 0.1081\ W^{5/8}$.

It should be noted that the values given in Tables 15 and 16 were obtained with no attempt to standardize the ration consumed previous to the fast and with no attempt to empty the intestinal tract of the steer by the use of cathartics, procedures used in the Pennsylvania experiments. The fasting heat production in the second experiment (Table 15), secured after a period of feeding at the one-fifth full feed level was, in fact, slightly greater per square meter of surface than the fasting heat production in the first experiment, secured after a period of feeding at the three-fifths full feed level. In the earlier series of experiments (Table 16) the steers were, previous to the fast, consuming a fattening ration of alfalfa hay and corn upon which they had been making gains of 2.49, 1.79, 1.36, and 1.54 pounds daily, respectively, for a period of 44 days. There appears to be no indication that the level of feeding previous to the fast has any appreciable influence on the heat production of fasting after the third day. This does not seem a matter of surprise to the writers, since, even though the paunch may still contain considerable food, its passage from the paunch into the third and fourth stomachs and

the intestinal tract would presumably be very slow, as would also be the rate of its digestion and absorption. Experiments referred to at the beginning of this paper, as well as the experiment now under discussion indicate clearly that the digestion and absorption of small amounts of food have only an inappreciable effect upon heat production.

In Table 15 will be found computations of the fasting heat production of the steer per 500 kg body weight, computed in the proportion of the 0.56 power of the weight. The six computations agree fairly well and average 8,503 Calories. This figure has been used in the computations of the fasting heat production of the steer during the five feeding periods (Table 17), in estimating the heat increment due to feeding. The surface area of the steer was not directly determined during these periods.

TABLE 17.—*Calculation of the heat increments and the net energy values of the rations*

Plane of nutrition	Dry matter consumed	Metabolizable energy intake	Observed heat production	Energy balance	Estimated heat production of fasting: Standard day	Activity increment	Total heat production of fasting	Heat increment due to feed	Heat increment per kilogram of dry matter consumed	Net energy		
										Total	Per kilogram of dry matter consumed	In percentage of metabolizable energy
	Kilograms	*Calories*	*Calories*	*Calories*	*Calories*	*Calories*	*Calories*	*Calories*	*Calories*	*Calories*	*Calories*	*Per cent*
Full feed	11.229	29,881	19,170	10,711	9,604	+180	9,784	9,386	836	20,495	1,825	68.6
	11.229	29,881	18,654	11,227	9,639	+10	9,649	9,005	802	20,876	1,859	69.9
Average	11.229	29,881	18,912	10,969	9,621	.....	9,717	9,195	819	20,685	1,842	69.3
Four-fifths	8.975	24,320	16,255	8,065	9,342	+334	9,676	6,579	733	17,741	1,977	72.9
	8.975	24,320	15,486	8,834	9,294	+60	9,354	6,132	683	18,188	2,027	74.8
	8.975	24,320	16,025	8,295	9,394	+257	9,651	6,374	710	17,946	2,000	73.8
Average	8.975	24,320	15,922	8,398	9,343	.....	9,560	6,361	709	17,958	2,001	73.8
Three-fifths	6.806	18,982	14,901	4,081	9,631	+376	10,007	4,894	719	14,088	2,070	74.2
	8,806	18,982	13,600	5,382	9,702	−293	9,409	4,191	616	14,791	2,173	77.9
	6,806	18,982	14,760	4,222	9,655	+68	9,723	5,037	740	13,945	2,049	73.5
Average	6.806	18,982	14,420	4,562	9,663	.....	9,713	4,707	692	14,275	2,097	75.2
Two fifths	4.644	13,563	12,387	1,176	9,545	−47	9,498	2,889	622	10,674	2,298	78.7
	4.644	13,563	12,559	1,004	9,496	−49	9,447	3,112	670	10,451	2,250	77.1
	4.644	13,563	12,610	953	9,549	−2	9,547	3,063	660	10,500	2,261	77.4
Average	4,644	13,563	12,519	1,044	9,530	.....	9,497	3,021	651	10,542	2,270	77.7
One-fifth	2.323	7,187	10,047	−2,860	9,436	+119	9,555	492	212	6,695	2,882	93.2
	2,323	7,187	9,548	−2,361	9,436	−67	9,369	179	77	7,008	3,017	97.5
	2.323	7,187	9,670	−2,483	9,436	−48	9,388	282	121	6,905	2,972	96.1
Average	2.323	7,187	9,755	−2,568	9,436	.....	9,437	318	137	6,869	2,957	95.6

The final results of the experiments are contained in Table 17. In the fourth column of this table will be found the observed heat production of the steer for 24 hours, computed as above explained. The difference between these values and the daily intakes of metabolizable energy yields the energy balances, which averaged for the different periods, in the order of decreasing food consumption, 10,969, 8,398, 4,562, 1,044, and −2,568 Calories. It appears from these

figures that the level of feeding equal to two-fifths full feed was slightly above the maintenance level. To obtain the heat increments, the fasting heat production (standard day) plus the estimated heat liberated due to standing for more than 12 hours must be deducted from the observed heat production; these values are given in columns 6, 7, and 8. The total heat increment and the increment computed to the kilogram of dry matter will be found in columns 9 and 10. The latter values for the five levels of feed are, in the order of decreasing food consumption, 819, 709, 692, 651, and 137 Calories. The net energy results are given in the last three columns. Per kilogram of dry matter consumed, the net energy values averaged, in the same order of feeding, 1,842, 2,001, 2,097, 2,270, and 2,957 Calories. The percentage availability of the metabolizable energy averaged 69.3 at the level of full feed, 73.8 at four-fifths full feed, 75.2 at three-fifths full feed, 77.7 at two-fifths full feed, and 95.6 at one-fifth full feed.

The biological error in this experiment is represented by the differences between the daily heat productions at each level of feeding. The average percentage difference between each two days' results at each of the five levels of feeding and of the two fasting periods was 3.02. If these observed heat productions are corrected to a standard day, by means of the activity increments given in column 7 of Table 17, the average difference becomes 2.29 per cent (standard deviation, 1.93), and in all but 5 of the 19 comparisons the correction for activity resulted in smaller differences. In two recent publications by Forbes and his associates (*9*, *10*), the average difference in observed daily heat productions on the same level of feeding, uncorrected to a standard day, was 1.92 per cent, standard deviation, 1.43. In the compilation of early experiments from the same laboratory in 1915, published by Armsby and Fries (*2*), the average percentage difference between duplicate determinations of daily heat production corrected to the standard day is 2.73, the standard deviation of the 75 differences being 2.19. In the consideration of the necessary degree of refinement in the technic of animal calorimetric investigations the extent of this inevitable biological error must find a place. It is of particular importance in assessing the significance of small differences in energy metabolism caused by differences in the plane of nutrition.

DISCUSSION OF RESULTS

The direct experimental measurement of the heat increment due to food is the difference between the observed heat production and the fasting heat production, corrected to equal body weight and, in so far as possible, to equal muscular activity. The use of lower levels of feeding in the computation of heat increments in place of the fasting condition gives values of uncertain significance. Their interpretation would seem to involve necessarily some particular theory concerning the factors responsible for the heating effect of food. For example, if the heat production on a maintenance ration is used in the computation of a heat increment between it and some higher level of feeding, the implication would seem to be that the excess feed above maintenance has itself suffered a loss in energy equal to this computed increment. It has been found that this increment per kilogram of dry matter consumed is greater than the

increment obtained between fasting and maintenance. The conclusion has therefore been drawn that the utilization of feed energy in fattening is less than that of maintenance. But this implies that the heating effect of food varies with the purpose for which it is used, and this follows only from one of a number of possible theories of the cause of this effect, namely, that it is due to heat liberated in the course of the chemical reactions of intermediary metabolism (*16*).

If the heat increment computed between maintenance and some higher level of feeding is not related only to the excess food, but also represents an increase in the heat loss suffered by the maintenance ration itself, then the increment possesses a mixed significance and can not logically be used, for example, in computing the net energy value of the ration for fattening only. In this case the heat increment might be the result entirely of the level of feeding and of the plethora of food nutrients set up in the blood and the intercellular fluids. The percentage utilization of the metabolizable energy would then suffer a decrease with increasing levels of feeding quite analogous to the concomitant decrease in the percentage digestibility of the food nutrients. Hence, it would be as illogical to compute a separate value for the net energy content of food consumed above maintenance as to compute separately its digestibility.

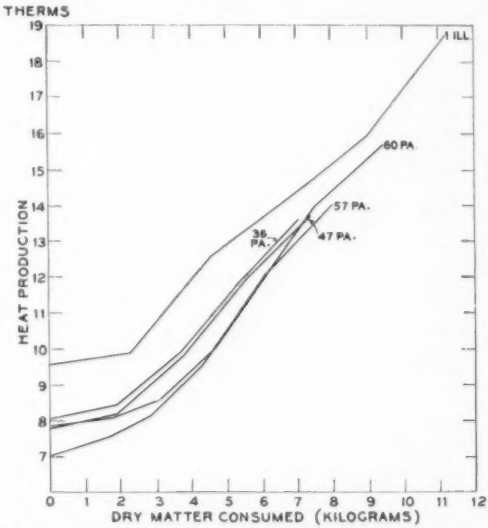

FIGURE 3.—Relation between heat production of steers and the quantity of dry matter consumed daily, illustrated by data from this experiment and from experiments of Forbes and his associates

Until the causes underlying the heating effect of food, including its specific dynamic action, are much more completely understood than they are at present, it would seem to be the preferable procedure to interpret the results of this experiment, and of others similar to it, in the most direct fashion possible, giving to the total heat increments and the corresponding net energy values their simplest interpretations.

In the discussion which follows, the results of this experiment on one steer will be compared with the results, obtained in similar experiments on four steers by Forbes and his associates (*9*, *10*). These are the only published experiments, in so far as the writers are aware, that have been concerned with the effect of the level of nutrition upon the energy metabolism of steers and upon their utilization of food energy.

In Figure 3 the relation between the heat production of the five steers, corrected to a constant body weight, and the dry matter consumed daily, is depicted graphically. The broken lines representing this relation are quite similar in their general trend. From the fasting condition to the lowest level of feeding, the heat production increases but slightly. From the lowest to the next feed level, representing for all steers approximately a maintenance ration, the heat production increases more rapidly. From these points to higher levels the heat production increases in an approximately linear fashion, the slight irregularities being apparently of no significance since they are not consistent. The intervals between the five lines are largely due to the differences in the body weights of the steers to which the heat productions have been corrected. The two lowest curves are for the two lightest steers, Nos. 57 Pa. and 60 Pa., with weights of 399 and 383 kg, respectively. The two intermediate curves are for the next heaviest steers, Nos. 36 Pa. and 47 Pa., weighing 468 and 479 kg, respectively. The heat production of the Illinois steer was corrected to the average weight of the two fasting periods, namely, 615 kg.

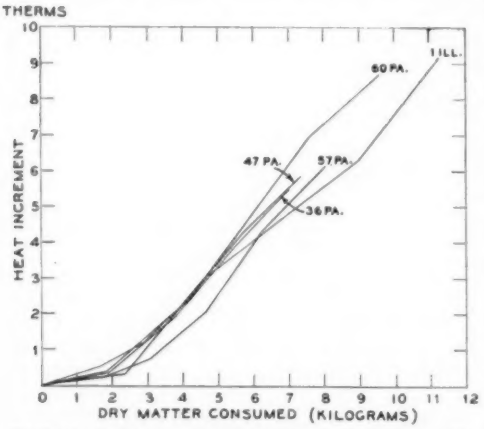

FIGURE 4.—Relation between total heat increment and quantity of dry matter consumed daily, illustrated by data from this experiment and from experiments of Forbes and his associates

The curves showing the relation between the total heat increments and the dry matter consumed (fig. 4) are much closer together except at the higher levels of feeding. The discrepancy here between steers 60 Pa. and 1 Ill., which were carried to higher levels of feeding than the others, may be due to individuality or possibly to the differences in ration, the ration of the Illinois steer containing more concentrates.

When the net energy values of the rations per kilogram of dry matter are plotted against the daily intake of dry matter, as in Figure 5, it appears that, except for the lowest levels of intake for steers 57 Pa., 60 Pa., and 1 Ill., the relation is more or less perfectly a linear one. The lines drawn upon this chart have been determined from the data of each steer (with the exceptions noted) by the method of least squares. The difference in slope between the line for the Illinois steer and the lines for the Pennsylvania steers may be due to the difference in the character of the rations fed.

The goodness of fit of these lines to the data of the five steers is indicated in Table 18, which contains the observed net energy values of the rations at the different levels of intake and the values estimated from the equations describing the straight lines, given in the last column of the table. For steers 47 Pa. and 36 Pa. it appears to be

possible to predict by linear equations the net energy value of the ration, for all levels of intake observed, with an error of less than 1 per cent. For steers 60 Pa. and 57 Pa., the predictions fall within 5 per cent of the observed values, with the exception of the results of the lowest levels of intake, which do not fall in line with the others and for which no predictions have been made. For the Illinois steer, again neglecting the result of the lowest level of feeding, the net energy value of the ration can be predicted within 1 per cent by the linear equation fitted to the data.

Although it might be supposed that the net energy value of a ration could be predicted considerably more accurately from the intake of metabolizable energy than from the intake of dry matter, this did not prove to be the case with steers 60 Pa. and 57 Pa., for which the dry matter net energy relation deviated the most from linearity. For the prediction on the basis of dry matter intake, the average percentage deviations between observed and predicted values for these two steers were 3.24 and 2.20, respectively. On the basis of the intake of metabolizable energy, the average percentage deviations between the observed net energy values and those predicted from a linear equation were 2.94 and 2.20, respectively. The same situation existed in the case of the other three steers.

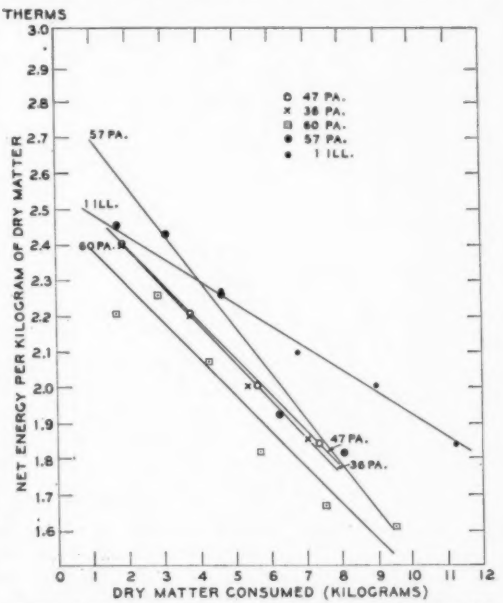

FIGURE 5.—The net energy value of various rations as affected by the quantity of dry matter consumed; illustrated by data from this experiment and from experiments of Forbes and his associates

It would appear that the problem of determining the net energy value of a ration has become more complicated as the result of the demonstration in a number of laboratories of the influence of the plane of nutrition. If the few results illustrating this influence in steers may be taken as a good indication of the truth, it seems that for each ration the net energy value may be expressed, not as a definite point in accordance with the Armsby system, but better as a linear equation relating the net energy value to the dry matter intake. Thus, from the Pennsylvania results, the net energy value of a ration consisting of equal parts of alfalfa hay and corn can not be assigned a definite value for all conditions of feeding, but may be predicted for all levels of feeding above maintenance from the linear equation $y=2.633-0.1108x$, in which x is the dry matter consumed in kilo-

grams and y is the net energy of the ration per kilogram of dry matter. The constants in this equation are the averages of the constants of the four equations for the individual steers. For low levels of nutrition (submaintenance (?)), the validity of a linear relation has not been established. More investigation needs to be done in clarifying the relation between net energy value and dry matter consumed at these levels.

Wiegner and Ghoneim (*21*) have described the relation between the intake of metabolizable energy and that of net energy, both expressed per square meter of body surface, by the differential equation

$$\frac{dA}{dF}=K(H-A)$$

in which A is the net energy intake, F the intake of metabolizable energy, H is the maximum value of A, and K is an efficiency constant.

TABLE 18.—*Prediction of net energy value of ration from the quantity of dry matter consumed, using Pennsylvania and Illinois data*

Steer No.	Dry matter consumed daily (x)	Net energy of ration per kilogram of dry matter		Difference	Prediction equation
		Observed	Calculated (y)		
	Kilogram	*Therms*	*Therms*	*Per cent*	
47 Pennsylvania	7.384	1.842	1.839	−0.2	$y=2.622-0.1060x$.
	5.617	2.012	2.027	+.7	
	3.790	2.241	2.220	−1.0	
	1.863	2.414	2.425	+.4	
36 Pennsylvania	7.037	1.857	1.857	0	$y=2.605-0.1065x$.
	5.353	2.027	2.035	+.4	
	3.762	2.218	2.204	−.7	
	1.885	2.399	2.404	+.2	
60 Pennsylvania	9.489	1.605	1.528	−4.8	$y=2.485-0.1009x$.
	7.520	1.667	1.726	+3.5	
	5.704	1.823	1.909	+4.7	
	4.237	2.071	2.057	−.7	
	2.828	2.257	2.200	−2.5	
57 Pennsylvania	8.057	1.818	1.774	−2.3	$y=2.821-0.1299x$.
	6.233	1.923	2.011	+4.6	
	4.612	2.260	2.222	−1.7	
	3.085	2.426	2.420	−.2	
1 Illinois	11.229	1.842	1.847	+.3	$y=2.538-0.0615x$.
	8.975	2.001	1.986	−.7	
	6.806	2.097	2.119	+1.0	
	4.644	2.266	2.252	−.6	

The integrated form of this equation, $1n\frac{H}{H-A}=KF$, was fitted to their own data on a rabbit and to the Pennsylvania data on four steers. In the latter case the average percentage deviation between observed and computed data was 3.9 and the maximum 11.7, one equation being used, i. e.,

$$1n\frac{5011}{5011-A}=0.0001995F$$

One may question the significance of reducing total intakes of energy to the square meter of body surface.

SUMMARY AND CONCLUSIONS

The energy metabolism of a grade Shorthorn steer, approximately 2 years of age, has been investigated at six levels of nutrition, ranging from fasting to full feed. The ration used was the same at all levels, containing on the dry matter basis 73.1 per cent of ground corn, 24.1 per cent of alfalfa hay, 2.0 per cent of linseed meal, and 0.8 per cent of molasses.

The lowest level of feeding was associated with the most complete digestibility of all nutrients. However, there was a progressive decrease in digestibility from the lowest to the highest ration only in the case of nitrogen-free extract, ether extract, and dry substance.

The metabolizable energy of the ration per kilogram of dry matter, as well as the percentage metabolizability of the gross energy, increased progressively from the highest to the lowest level of feeding, the former from 2,661 Calories to 3,094 Calories, and the latter from 60.61 per cent to 70.14 per cent. However, when the metabolizable energy is computed to a kilogram of digestible nutrients, all effect of the level of feeding disappears.

At the two highest levels of feeding, the respiratory quotient was continuously greater than 1 for the entire day. At all levels of feeding except the lowest, the respiratory quotient for the first four hours after feeding was higher than for any other period. The average daily respiratory quotients for the five levels of feeding in the order of decreasing levels were 1.13, 1.12, 1.05, 0.97, and 0.88.

The average fasting heat production of the steer, corrected to a standard day of 12 hours in the standing position and 12 hours in the lying position, averaged 1,858 Calories per square meter of body surface, as directly determined with the Brody surface integrator. If the surface area of the steer is computed by the Moulton formula, based upon skin areas, a much larger value is obtained than that actually observed, and the fasting heat production per square meter becomes 1,554 Calories per day, a value consistent with previously published values for steers. Serious discrepancies exist between formulas now being used for the estimation of the surface area of steers.

The average of 13 determinations of the fasting heat production of beef cattle, corrected to the standard day, from the Illinois and Pennsylvania experiments, is $1,897 \pm 19$ Calories per square meter of body surface, the latter computed according to the Brody formula. The standard deviation of these 13 determinations was 100 and their coefficient of variation 5.27.

In this experiment, although one fasting period followed a three-fifths full-feed period and one a one-fifth full-feed period, no evidence was obtained that the preceding feeding affects appreciably the heat production of the fourth, fifth, and sixth days of fast. Other experiments performed in this laboratory are cited to the same effect.

The heat increment due to feeding, per kilogram of dry matter consumed, increased from the lowest to the highest levels of feeding, while the net energy values, on the same basis, decreased progressively from 2,957 Calories to 1,842 Calories. The percentage availability of the metabolizable energy assumed the following values from the lowest to the highest levels of feeding: 95.6, 77.7, 75.2, 73.8, and 69.3. This decrease in total utilization of feed by steers seems to be due in

part to a more or less continuous decrease in digestibility, but also to a very slight specific dynamic effect of feed at very low levels of feeding.

From the results of this experiment and of the experiments on the same problem published by Forbes and his associates, it appears that, except for submaintenance levels of feeding, the net energy value of a ration bears a linear relation to the amount of dry matter consumed. Hence, the net energy value of a ration may be defined, not by a constant, as Armsby supposed, but by a linear equation relating it to the intake of dry matter. There are grounds for suspecting that the size of steer may eventually have to be considered also in defining this relation, but at present the data are not at hand to decide this question.

LITERATURE CITED

(1) Andersen, A. C.
1922. zur ausführung und berechnung von stoffwechselversuchen mit wiederkäuern. Biochem. Ztschr. 130 : 143–150.

(2) Armsby, H. P., and Fries, J. A.
1915. net energy values of feeding stuffs for cattle. Jour. Agr. Research 3 : 435–491, illus.

(3) Benedict, C. G., and Benedict, F. G.
1923. a permissible breakfast prior to basal metabolism measurements. Boston Med. and Surg. Jour. 188 : 849–851.

(4) Benedict, F. G., and Carpenter, T. M.
1918. food ingestion and energy transformations, with special reference to the stimulating effect of nutrients. 355 p., illus. Washington, D. C. (Carnegie Inst. Wash. Pub. 261.)

(5) ——— and Joslin, E. P.
1910. metabolism in diabetes mellitus. 234 p., illus. Washington, D. C. (Carnegie Inst. Wash. Pub. 136.)

(6) ——— and Ritzman, E. G.
1927. the metabolism of the fasting steer. 246 p., illus. Washington, D. C. (Carnegie Inst. Wash. Pub. 377.)

(7) Brody, S., and Elting, E. C.
1926. growth and development with special reference to domestic animals. II. a new method for measuring the surface area and its utilization to determine the relation between growth in surface area and growth in weight and skeletal growth in dairy cattle. Missouri Agr. Expt. Sta. Research Bul. 89, 18 p., illus.

(8) Carpenter, T. M., Fox, E. L., and Sereque, A. F.
1929. the carpenter form of the haldans gas analysis apparatus. changes made in the apparatus and details regarding its use. Jour. Biol. Chem. 83 : 211–230, illus.

(9) Forbes, E. B., Braman, W. W., and Kriss, M., with the collaboration of Jeffries, C. D., Swift, R. W., French, R. B., Miller, R. C., and Smythe, C. V.
1928. the energy metabolism of cattle in relation to the plane of nutrition. Jour. Agr. Research 37 : 253–300, illus.

(10) ——— Braman, W. W., and Kriss, M., with the collaboration of Swift, R. W., French, R. B., Smythe, C. V., Williams, P. S., and Williams, H. H.
1930. further studies of the energy metabolism of cattle in relation to the plane of nutrition. Jour. Agr. Research 40 : 37–78, illus.

(11) ——— Fries, J. A., Braman, W. W., and Kriss, M.
1926. the fasting katabolism of dry cows. Jour. Agr. Research 33 : 591–595.

(12) ——— Kriss, M., and Braman, W. W.
1927. the computed as compared with the directly observed fasting katabolism of cattle as a measure of the maintenance requirement of energy. Jour. Agr. Research 34 : 167–179.

(13) Hogan, A. G., and Skouby, C. I.
1923. DETERMINATION OF THE SURFACE AREA OF CATTLE AND SWINE. Jour. Agr. Research 25: 419–430, illus.

(14) Lusk, G.
1928. THE ELEMENTS OF THE SCIENCE OF NUTRITION. Ed. 4, reset, 844 p., illus. Philadelphia and London.

(15) Magnus-Levy, A.
1907. THE PHYSIOLOGY OF METABOLISM. Anglo-Amer. issue under the editorship of I. W. Hall. 452 p. Chicago. *In* Noorden, C. von, Metabolism and Practical Medicine, v. 1.

(16) Mitchell, H. H.
1927. DOES THE NET ENERGY VALUE OF FOOD DEPEND UPON THE PURPOSE FOR WHICH IT IS USED IN THE BODY? Science (n. s.) 66 : 289–292.

(17) Møllgaard, H.
1929. FÜTTERUNGSLEHRE DES MILCHVIEHS. DIE QUANTITATIVE STOFFWECHSELMESSUNG UND IHRER BISHERIGEN RESULTATE BEIM MILCHVIEH. 246 p., illus. Hannover.

(18) Moulton, C. R.
1916. UNITS OF REFERENCE FOR BASAL METABOLISM AND THEIR INTERRELATIONS. Jour. Biol. Chem. 24 : 299–320, illus.

(19) Ritzman, E. G., and Benedict, F. G.
1929. SIMPLIFIED TECHNIQUE AND APPARATUS FOR MEASURING ENERGY REQUIREMENTS OF CATTLE. N. H. Agr. Expt. Sta. Bul. 240, 30 p., illus.

(20) Soderstrom, G. F., Barr, D. P., and Du Bois, E. F.
1918. CLINICAL CALORIMETRY. TWENTY-SIXTH PAPER. THE EFFECT OF A SMALL BREAKFAST ON HEAT PRODUCTION. Arch. Int. Med. 21 : 613–620, illus.

(21) Wiegner, G., and Ghoneim, A.
1930. ÜBER DIE FORMULIERUNG DER FUTTERWIRKUNG. EIN BEITRAG ZUR THEORIE DER VERWERTUNG DES UNTERERNÄHRUNGS- UND PRODUKTIONSFUTTERS AUF GRUND VON NEUEN FÜTTERUNGSVERSUCHEN. Tierernährung 2 : 193–232, illus.

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